OXYGEN UPTAKE KINETICS DURING EXERCISE

by

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Abstract

This thesis has investigated oxygen uptake ($\dot{V}O_2$) kinetic regulation during whole body exercise by investigating not only the breath-by-breath $\dot{V}O_2$ response to a change in work rate but also incorporating an examination of data analysis processes and simulation of a physiological $\dot{V}O_2$ model. Five experiments have been completed to answer the following questions:

Experiment 1: What effects do sample duration, ensembling, interpolation, and smoothing have on time constant determination?

Experiment 2: Is the slope of the $\dot{V}O_2$ response to a ramp work rate altered by changing the inspired fraction of oxygen?

Experiment 3: Does glycogen depletion acutely reduce the rate of carbohydrate utilization and thus quicken the $\dot{V}O_2$ time constant?

Experiment 4: Does one-legged training alter the $\dot{V}O_2$ time constant of the trained and the untrained leg differently?

Experiment 5: With respect to the muscle $\dot{V}O_2$ time constant, does expansion of an accepted physiological model of oxygen uptake to include a heterogeneous muscle compartment significantly alter modeled $\dot{V}O_2$ kinetics?

Experiment 1 demonstrated that a more accurate $\dot{V}O_2$ time constant estimation from a step increase in work rate can be acquired by artificially extending the steady state portion of the $\dot{V}O_2$ response. Experiment 2 showed that, above the anaerobic threshold, the $\Delta\dot{V}O_2/\Delta WR$ relation, relative to normoxia, is increased in hyperoxia and decreased in hypoxia. This suggests that a change in $\Delta\dot{V}O_2/\Delta WR$ above the anaerobic threshold is related to the recruitment pattern of muscle units and their kinetic characteristics rather than simply the onset of anaerobiosis. In experiment 3 there was no difference in the $\dot{V}O_2$ kinetic response of the glycogen-depleted state compared with a normal glycogen state. This indicates that neither the physiological nor biochemical changes induced by glycogen depletion affected $\dot{V}O_2$ kinetics. The trained leg in experiment 4 had a faster $\dot{V}O_2$ response than the untrained leg. A simple conclusion from this is that $\dot{V}O_2$ kinetics are

regulated peripherally. However, the heart rate response also displayed an interaction with training thus confounding any simple interpretation. The computer simulation of experiment 5 illustrated that when a whole muscle is subcompartmentalized into individual muscle units, each with a different $\dot{V}O_2$ time constant, the pulmonary $\dot{V}O_2$ response is still exponential. Furthermore, the slow $\dot{V}O_2$ increase observed above the anaerobic threshold cannot be attributed to a continuous variation in muscle unit time constant.

Individual findings from this thesis may support either a central or peripheral locus of $\dot{V}O_2$ kinetic regulation. Overall the conclusion must be different however. An argument is developed that for most physiological conditions, it is the interplay of both central **and** peripheral processes that establish the rate at which O_2 diffuses into working muscle in response to an increase in work rate.

Dedication

I dedicate this thesis to my wife, Janice Walsh and my parents, Muriel and Leonard Walsh. Without their support and love this thesis would not have been possible.

Acknowledgments

First, I would like to thank all the subjects for participating in these studies. Without their participation, I could not have done this thesis. The sacrifices they made for my data collection illustrate the high quality of those people.

Some of the equipment used for data collection required to be designed and manufactured on campus. For this I wish to thank Robert Taylor for his work on the inspired and expired flow switches, modification of equipment to determine expiratory flow rate and inspired volume, the heating of the gas sample line, creating a Turbo C program to run the cycle ergometer, and a whole bunch of other little things. I also thank Gavril Moriu and Colin Shaw for their work on the expired flow switch and I thank Victor Stobbs for always helping when help was needed.

I would like to thank my supervisor, Dr Eric Banister for his direction and unwavering support throughout my studies and research.

I would like to thank the fellow grad student who helped me the most, Barbara Cameron for her suggestions about the breath-by-breath $\dot{V}O_2$ algorithm, her work in helping to calibrate the whole system, and for glycogen-depleting some of my subjects. I wish to also thank Ian Dunsmiur, Douglas Dray, and Daniel Robinson for their help in calibrating the $\dot{V}O_2$ system.

Most of all I would like to thank my family: My loving wife Janice Walsh for her support and patience and to my parents Muriel and Leonard Walsh for their love and support.

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Abbreviations

A/D analogue/digital

ADP Adenosine diphosphate

AMP Adenosine monophosphate

ATP Adenosine triphosphate

B-by-B Breath by breath

C Creatine

Ca^{+2} Calcium

CaO_2 Concentration of O_2 in arterial blood ($ml \cdot 100\ ml^{-1}$)

CO_2 Carbon dioxide

CvO_2 Concentration of O_2 in venous blood ($ml \cdot 100\ ml^{-1}$)

CP Creatine phosphate

D/A Digital/analogue

$\Delta\dot{Q}(ss)$ The change in cardiac output ($ml \cdot min^{-1}$) from baseline to steady state

$\Delta\dot{V}O_2(ss)$ The difference in oxygen uptake between baseline and steady state

$\Delta\dot{V}O_2/\Delta WR$ The change in oxygen uptake per change in work rate

EMG Electromyography

$FECO_2$ Expired fraction of carbon dioxide

FEO_2 Expired fraction of oxygen

$FETCO_2$ End tidal fraction of carbon dioxide (%)

$FETO_2$ End tidal fraction of oxygen level (inverted %)

FIO_2 Inspired fraction of oxygen

FRC Functional residual capacity(l: BTPS)

H_2O Water

Hb Hemoglobin

HR Heart Rate (beats·min^{-1})

IEMG Integrated electromyograph

IMP Inosine monophosphate

MeanPF Mean power frequency

MedPF Median power frequency

N_2 Nitrogen

NAD^+ Nicotine adenine dinucleotide in oxidized state

NADH Nicotine adenine dinucleotide in reduced state

NMR Nuclear magnetic resonance

O_2 Oxygen

ON $\dot{V}O_2$ Oxygen uptake response to an increase in work rate

PcO_2 Partial pressure of oxygen in the capillary

Pi Inorganic phosphate

PmO_2 Partial pressure of oxygen in the mitochondrion

PO_2 Partial pressure of oxygen

$\dot{Q}O_2$ Oxygen uptake of a muscle

$\dot{Q}$ Cardiac output rate

RGET Respiratory gas exchange threshold

SaO_2 Saturation of hemoglobin with oxygen

SDH succinate dehydrogenase

τ Tau, the time constant. The time it takes to reach 63 % of steady state

$\tau_m\dot{V}O_2$ Time constant of O_2 uptake by the muscle comp.. tment

$\tau\dot{Q}$ Time constant of cardiac output

$t_{1/2}$ The time it take to reach 50 % of the difference between baseline and a new steady state condition

TCA Tricarboxylic acid

TLT Total lag time. Consist of the delay time plus the time constant.

$\dot{T}O_2$ Rate of oxygen transported

TE Expiration time (ms)

TT Total breath time (ms)

VA Alveolar ventilation (l: BTPS)

$\dot{V}CO_2$ Carbon dioxide elimination ($l \cdot min^{-1}$: STPD)

VD Dead space volume (l)

$\dot{V}E$ Expired ventilatory rate ($l \cdot min^{-1}$: BTPS)

$\dot{V}EO_2$ Rate of oxygen expired ($l \cdot min^{-1}$)

VI Inspired ventilation(l: BTPS)

$\dot{V}IO_2$ Rate of oxygen inspired ($l \cdot min^{-1}$)

$\dot{V}O_2$ Oxygen uptake ($l \cdot min^{-1}$: STPD)

$\dot{V}O_2$ tau The time constant of the $\dot{V}O_2$ response

$\dot{V}O_2$max Maximum oxygen uptake

W Watts

WR Work rate (Watts)

ZC Zero crossings

1.0 PROPOSED EXPERIMENTS

The determination of the oxygen uptake response ($\dot{V}O_2$) to changes in work rate provides information important to both the clinical and research fields of respiratory, cardiovascular, and muscular physiology. Investigation of the $\dot{V}O_2$ kinetic response to changes in energy demand is a young science discipline. As such, questions to be investigated concern not only the underlying physiology, but also other areas such as methodological procedures and simulation models. This thesis will investigate perturbations to the $\dot{V}O_2$ kinetic response to further our understanding of oxygen kinetics. In addition, investigations will also be conducted in the areas of methodological procedures and simulation models so as to better comprehend experiments that investigate the physiology of oxygen kinetics.

This thesis proposes to examine how the $\dot{V}O_2$ kinetic response is regulated. Breath-by-breath $\dot{V}O_2$ data collection and mathematical modeling will be the main tools used for these investigations. The questions addressed by the thesis proposal include:

1. Experiment 1: What effects do sample duration, ensembling, interpolation, and smoothing have on time constant determination?

2. Experiment 2: Is the slope of the $\dot{V}O_2$ response to a ramp work rate altered by changing the inspired fraction of oxygen?

3. Experiment 3: Does one-legged training alter the $\dot{V}O_2$ time constant of the trained and the untrained leg differently?

4. Experiment 4: Does glycogen depletion acutely reduce the rate of carbohydrate utilization and thus quicken the $\dot{V}O_2$ time constant?

5. Experiment 5: With respect to the oxygen uptake time constant, does a heterogeneous muscle compartment significantly alter modeled $\dot{V}O_2$ kinetics?

2.0 OXYGEN UPTAKE KINETICS-A GENERAL REVIEW

2.1 HISTORY

Exercise physiology attained prominence in the 1920's with the classical work of A.V. Hill. Although Hill used only protocols such as running on the spot and stepping up and down, his data were surprisingly accurate. Hill *et al.* (1924) were the first to describe the time course oxygen consumption ($\dot{V}O_2$) to a reduction in work rate as exponential.

$$A = Ao \cdot e^{-kt} \quad \text{.....(2-1)}$$

A is the oxygen consumption ($l \cdot min^{-1}$) at time, t. Ao is the difference between the starting and the end steady state O_2 consumption ($l \cdot min^{-1}$). k is the rate constant and is the inverse of the time constant (Tau=τ).

Hill applied Eqn. (2-1) to recovery $\dot{V}O_2$ curves only. The ON $\dot{V}O_2$ response was derived as an exponential process independently by Simonson (1927) and Henry (1951).

$$dy/dt = a_o \cdot (1 - e^{-kt}) \quad \text{.....(2-2)}$$

Transcribed to a more familiar construction, equation 2 becomes:

$$\Delta\dot{V}O_2(t) = \Delta\dot{V}O_2(ss) \cdot (1 - e^{-t/\tau}) \quad \text{.....(2-3)}$$

$\Delta\dot{V}O_2(t)$ ($l \cdot min^{-1}$) is the $\dot{V}O_2$ response at any time (t) above the baseline value and $\Delta\dot{V}O_2(ss)$ ($l \cdot min^{-1}$) is the difference between steady-state $\dot{V}O_2$ and the same baseline $\dot{V}O_2$ value.

Detailed examination of the $\dot{V}O_2$ kinetic response prior to the 1970's was limited by the number of samples that could be collected within a given time course. In the 1970's, improved technology enabled three major changes to occur which were material to the investigation of the transient kinetics of $\dot{V}O_2$ at the onset of exercise.

1) Technological advances in the area of gas analysis enabled gas analysis to be done on a breath-by-breath basis.
2) Improved computer technology enabled performance of the thousands of tedious calculations necessary for real time measurement of $\dot{V}O_2$ to be rapidly made. This allows

much more detailed analyses of collected data (*e.g.*, curve fitting with least squares algorithms and data averaging).

3) There has also been major progress in the ability of the investigator to apply a work rate (WR) with a wide variety of shape (*e.g.*, a step, ramp, or sinusoidal WR).

These three changes have allowed characterization of $\dot{V}O_2$ kinetics to a much greater degree of accuracy. Despite this enhanced analytical ability, controversy surrounding $\dot{V}O_2$ kinetics has also increased.

2.2 THE STEP WORK RATE STIMULUS

The $\dot{V}O_2$ response to a step increase in work rate is commonly represented by the graph shown in Fig. 2-1. For this work rate protocol the work rate increases 'instantaneously' to a new constant level. Thus the total rate of ATP production demanded by the work rate also increases 'instantaneously' to a new constant level. The $\dot{V}O_2$ response represents the

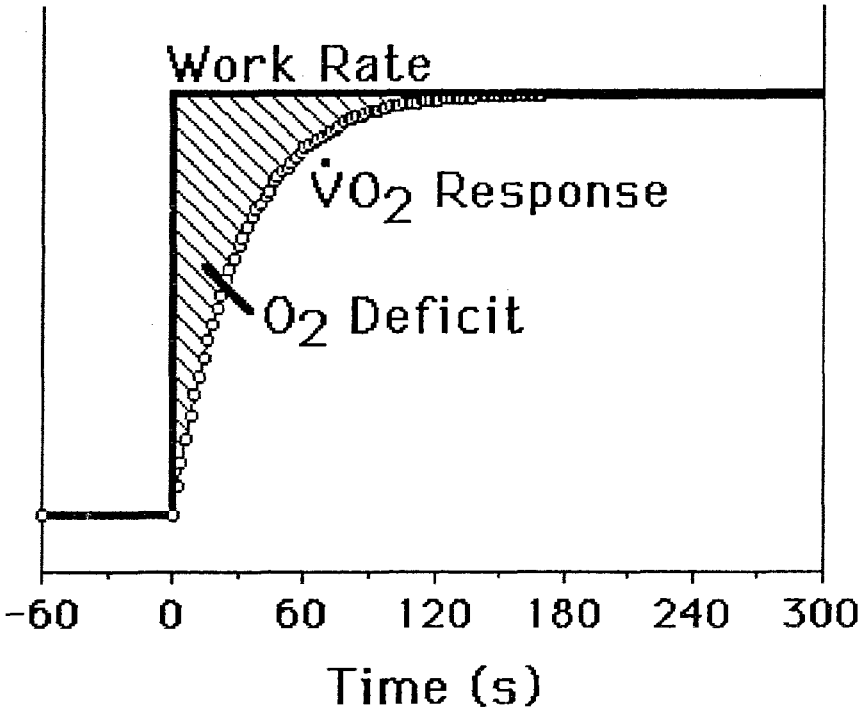

Fig. 2-1. The $\dot{V}O_2$ response to a step increase in work rate. The O_2 deficit is represented as the shaded area.

aerobic portion of the ATP production rate. The anaerobic portion of the ATP production rate is the difference of the total ATP production rate demanded by the work rate minus the aerobic production rate. The ON $\dot{V}O_2$ response to a step increase in work rate above a base line level of activity has most frequently been described mathematically by a single exponential function (Eqn. 2-3).

However, the $\dot{V}O_2$ response often exhibits three phases. Phase 1 is a rapid response lasting 10-20 s and has been attributed to cardiovascular origins (Fig. 2-2) (Krogh and Lindhard, 1913; Guyton *et al.*, 1962; Whipp, 1987).

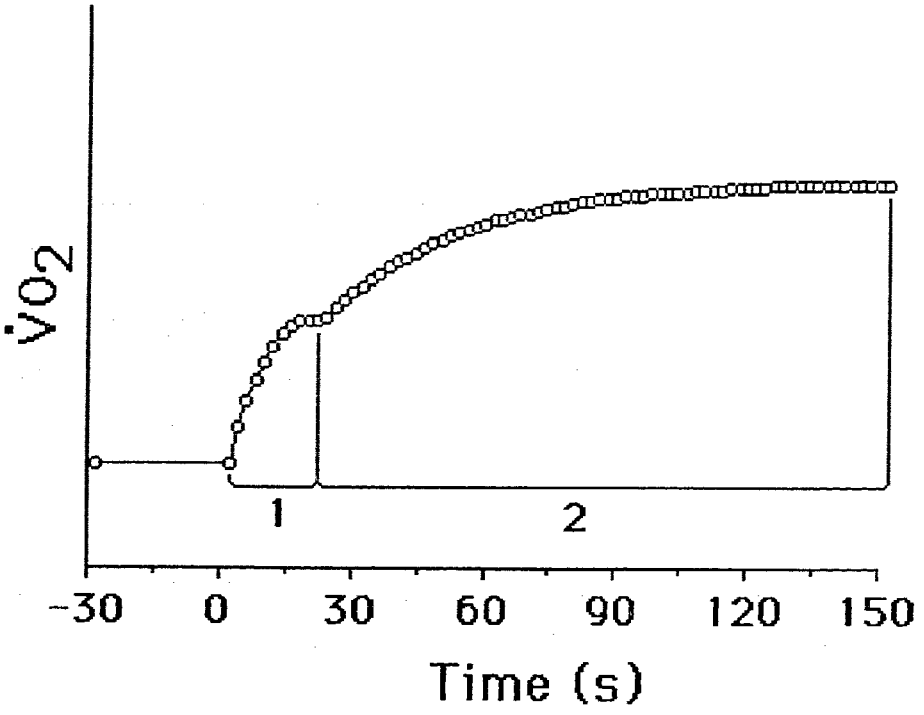

Fig. 2-2. Phase 1 and 2 of the $\dot{V}O_2$ response to a step increase in work rate.

Phase 1 is more prominent when a step change in work rate is initiated from rest rather than from a light work rate. Phase 2 is the $\dot{V}O_2$ rise to steady state. The exponential increase in $\dot{V}O_2$ in phase 2 has been attributed both to central (delivery) mechanisms and peripheral (diffusive) mechanisms (see section 6 for a discussion). Phase 3 represents the attainment of steady-state (Fig. 2-3). The start of phase 3 is subject to interpretation. It is reasonable to consider phase 3 as occurring after a

period equivalent to 5 time constants of the initial phase 2 response has elapsed (Stanley, 1968). In addition, there is a phase 4 response. It is observed when the amplitude of the work rate is greater than the anaerobic threshold. Phase 4 consists of a prolonged slow rise of $\dot{V}O_2$ in phase 3 that may or may not attain a steady state. Phase 4 represents an additional O_2 cost of exercise above that predicted by the steady state relation $\dot{V}O_2$ with work rate established by step changes to work rates below the anaerobic threshold (Roston *et al.*, 1987; Whipp, 1987). At a work rate above the anaerobic threshold, the $\dot{V}O_2$ response is better fitted by a double exponential with a delayed onset of the second exponential by 1-6 minutes, than by a single or double exponential with no time delay (Barstow and Mole, 1991; Casaburi and Wasserman, 1986; Paterson and Whipp, 1987; Paterson and Whipp, 1991).

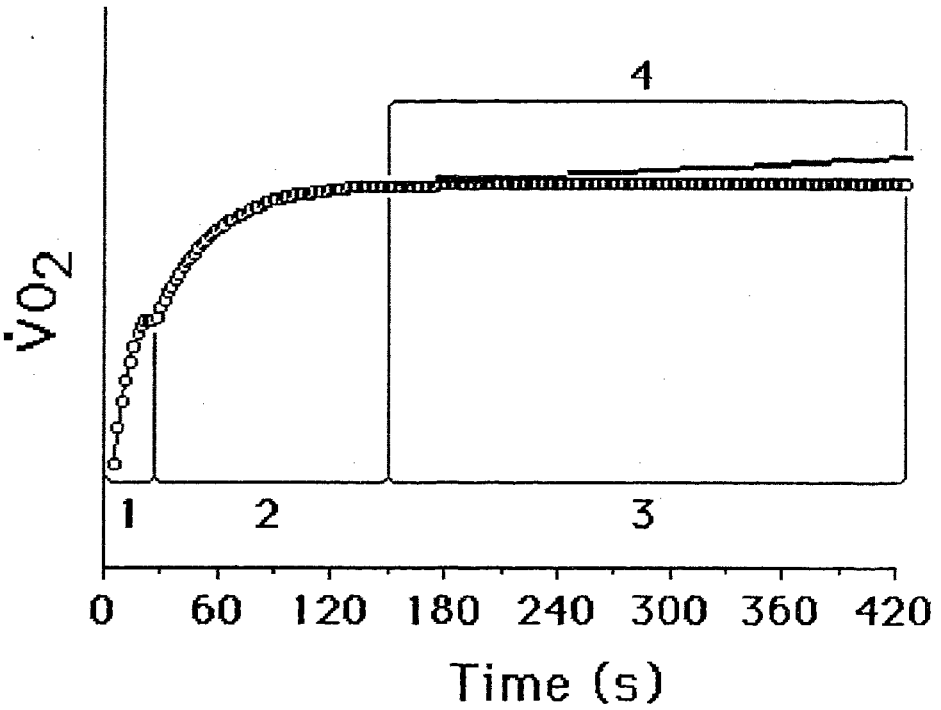

Fig. 2-3. $\dot{V}O_2$ response to a step increase in work rate. If the step increase is to a work rate below anaerobic threshold, phases 1, 2, and 3 are characteristically observed. If the step increase is to a work rate above the anaerobic threshold then the phase 3 response is replaced by the phase 4 curve.

2.2.1 OXYGEN DEFICIT OF THE STEP FUNCTION

The O_2 deficit (litres) is defined as the difference between $\Delta\dot{V}O_2(ss)$ and the actual cumulative $\dot{V}O_2$ (Fig. 2-1). Mathematically, the O_2 deficit is calculated from:

$$O_2 \text{ deficit} = \Delta\dot{V}O_2(ss) \cdot \tau - \Delta\dot{V}O_2(ss) \cdot \int_0^t (1 - e^{-t/\tau})\, dt \quad \text{.....(2-4)}$$

When $t \gg \tau$, the O_2 deficit is equivalent to

$$O_2 \text{ deficit} = \Delta\dot{V}O_2(ss) \cdot \tau \text{.....(2-5)}$$

The O_2 deficit has been attributed to ATP from alactic and lactic sources (Sahlin *et al.*, 1988; Whipp, 1971) supplementing energy requirements while the supply rate of oxidative ATP is inadequate. The O_2 deficit is not considered to be due to a hypoxic or anoxic condition within the muscle during the transition from rest to exercise (Connett, 1986; Gayeski *et al.*, 1985). The equivalency of the work rate and $\dot{V}O_2(ss)$ in Fig. 2-1 is commonly deduced by simple graphic manipulation rather than by systematic determination or calculation. By definition, the O_2

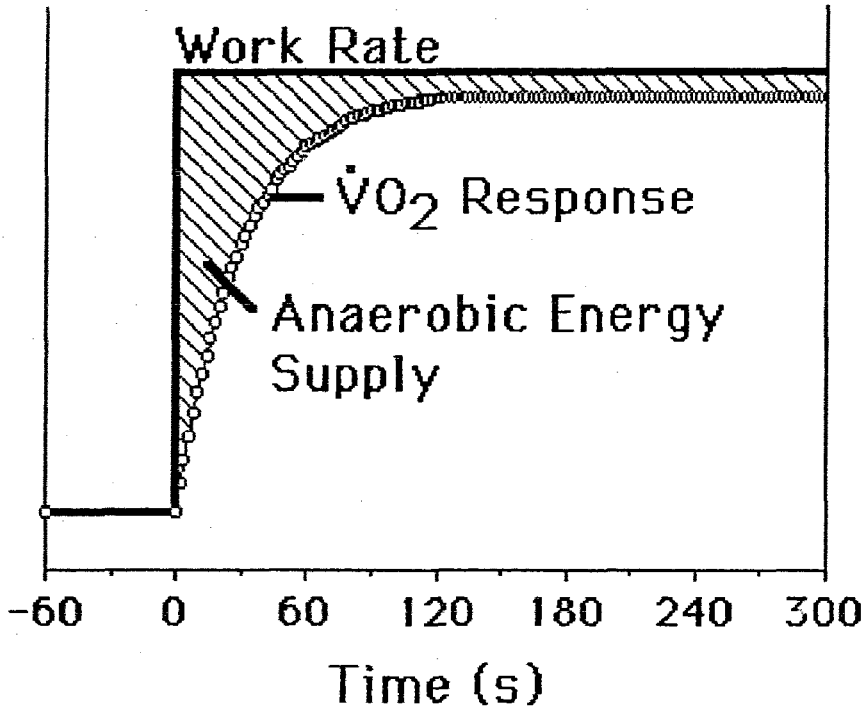

Fig. 2-4. Schematic representation of the $\dot{V}O_2$ response relative to a step increase in work rate.

deficit no longer changes once $\Delta\dot{V}O_2(ss)$ is attained. From an energetic perspective, this is incorrect. Glycolytic activity, with the final product being pyruvate and/or lactate, directly contributes high energy phosphate to the ATP pool during a steady state condition (Depocas *et al.*, 1969; Green *et al.*, 1983; Jorfeldt, 1970; Stanley *et al.*, 1985). Thus the $\dot{V}O_2$ response to a step increase in work rate should appear more like that shown in Fig. 2-4 than as in Fig. 2-1.

As in Fig. 2-1, Fig. 2-4 indicates that, in the steady state, the $\dot{V}O_2$ response and thus the aerobic production rate of ATP is constant. It also illustrates that in phase 3 a small amount of ATP is being produced by the glycolytic pathway. This anaerobically-produced ATP comes from glycolysis.

2.3 THE RAMP WORK RATE STIMULUS

The ramp function is commonly represented by the graph shown in Fig. 2-5. The work rate increases in a linear fashion and thus the rate of ATP production demanded by the work rate also increases in a linear manner.

Mathematically, the $\dot{V}O_2$ response to a ramp WR protocol is defined by:

$$\Delta\dot{V}o_2(t) = \Delta\dot{V}o_2(ss) \cdot (t - \tau \cdot (1 - e^{-t/\tau})) \quad \text{.....(2-6)}$$

In this equation $\Delta\dot{V}O_2(ss)$ is actually an acceleration term ($l \cdot min^{-2}$) representing the O_2 cost of the increment of the ramp slope (*i.e.*, 1 Watt per second is an acceleration since a Watt is a Joule per second).

The $\dot{V}O_2$ response shown in Fig. 2-5 is linear after a period of 5 time constants has elapsed. The graph axes are manipulated so that the work rate demand parallels the $\dot{V}O_2$ response. Tau $\dot{V}O_2$ can be determined by extending a line from the linear portion of the $\dot{V}O_2$ response downward to the time axis. The time from initiation of the work rate to the baseline intersection of the extrapolated linear $\dot{V}O_2$ response then represents the time constant for $\dot{V}O_2$ (Fig. 2-6) (Davis *et*

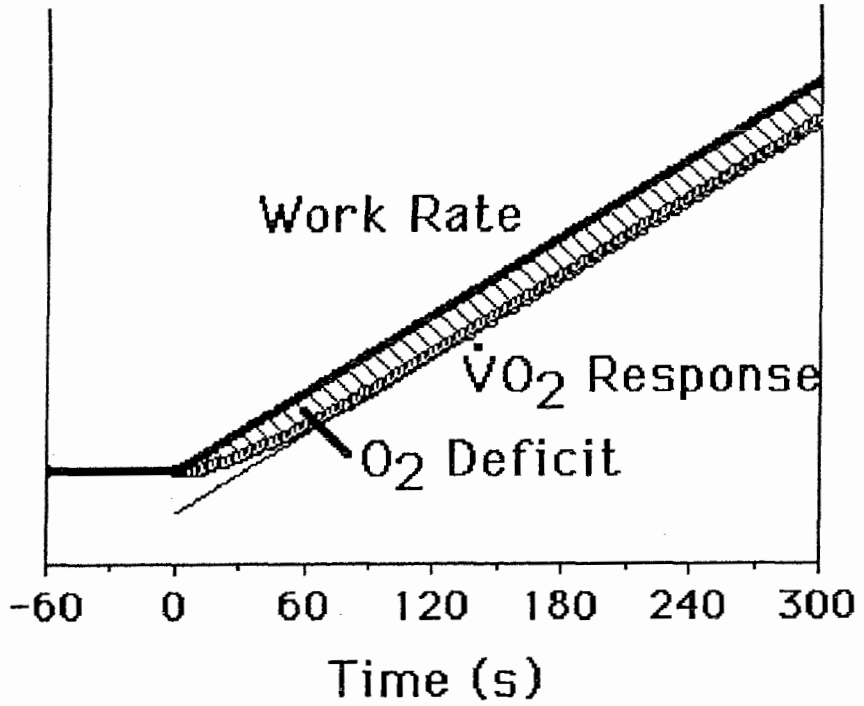

Fig. 2-5. $\dot{V}O_2$ response induced by a ramp increase in work rate. The O_2 deficit is represented by the shaded area.

al., 1982). Defining the $\dot{V}O_2$ response as a linear function of work rate demand implies that tau $\dot{V}O_2$ is the same for all work rates (Fig. 2-5 and 2-6). This is a controversial point addressed in section 5.

Hansen *et al.* (1988) found that for work rate ramp increments of 15, 30, and 60 $W \cdot min^{-1}$, the mean slopes for the entire $\dot{V}O_2$ response except the end portions were 11.2, 10.2, and 8.8 $ml \cdot min^{-1} \cdot W^{-1}$, respectively. The slopes of the lower halves of the $\dot{V}O_2$ response to the 15 and 30 $W \cdot min^{-1}$ work rate increments were 9.9 $ml \cdot min^{-1} \cdot W^{-1}$. However the upper halves of the $\dot{V}O_2$ response to 15, 30, and 60 $W \cdot min^{-1}$ work rate ramps were significantly different: 12.4, 10.5, and 8.7 $ml \cdot min^{-1} \cdot W^{-1}$, respectively. The authors speculated that the lower $\Delta\dot{V}O_2/\Delta WR$ at 60 $W \cdot min^{-1}$ is due to a major anaerobic energy contribution over a short period of time. The increase in slope in the second half of the 15 $W \cdot min^{-1}$ test is due to the increased O_2 cost of heavier exercise. And the maintained linearity of the 30 $W \cdot min^{-1}$ $\Delta\dot{V}O_2/\Delta WR$ response is due to a fortuitous cancelling of these opposing effects.

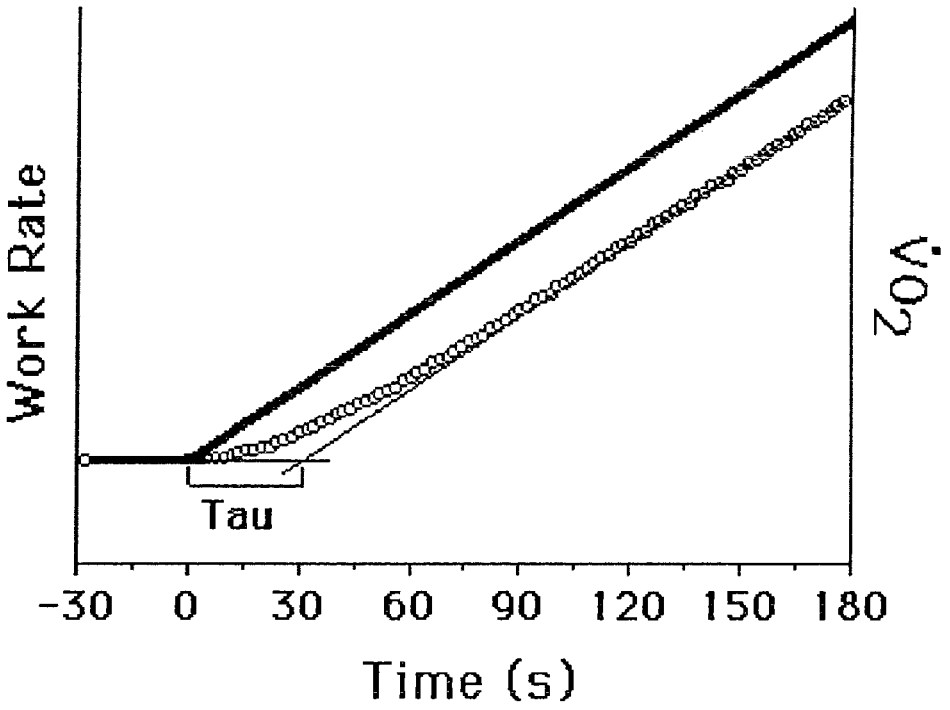

Fig. 2-6. Graphical determination of the $\dot{V}O_2$ (open circles) time constant (tau) when the work rate (solid line) function is a ramp increment.

2.3.1 O_2 DEFICIT OF THE RAMP FUNCTION

The ramp function generally implies that the slope of the work rate induced ATP production rate is equivalent to the slope of the $\dot{V}O_2$ response. This assumption is questionable. As pointed out for the step function, ATP production rate required for a given WR is supplied from both aerobic and anaerobic processes. Logically, as ATP demand increases one should expect glycolytic flux to increase at least enough to maintain a constant proportional contribution to the total ATP energetic demand. This implies that the slope of the work rate induced ATP production diverges from the slope of the $\dot{V}O_2$ response. The generally accepted version of ramp function $\dot{V}O_2$ kinetics indicates that as the ATP demand increases at a higher work rate, the O_2 deficit contribution to the total ATP production rate remains constant and is not proportional to the work

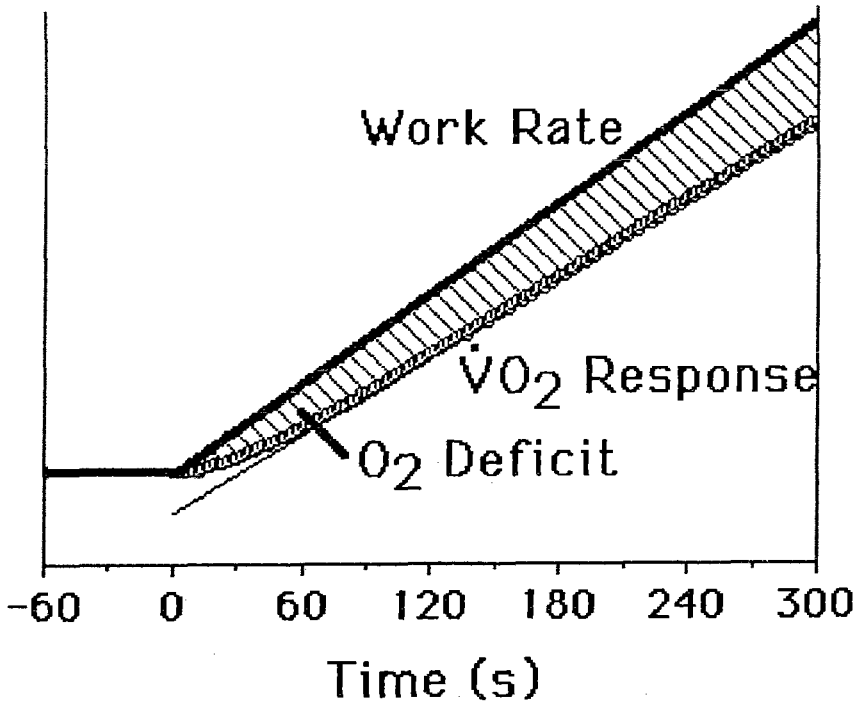

Fig. 2-7. A schematic representation of an alternative relation of $\dot{V}O_2$ to work rate during a ramp protocol.

rate demand (Fig. 2-5 and 2-6). An alternative rationale is diagrammed in Fig. 2-7. This figure indicates that as work rate increases so does the ATP contribution from O_2 deficit sources as well as from aerobic sources. The $\dot{V}O_2$ kinetics illustrated in Fig. 2-7 assumes that as work rate increases so does the time constant of the $\dot{V}O_2$ response.

3.0 COMMON EXPERIMENTAL METHODOLOGY

Data collection and analysis procedures common to all proposed experiments are outlined below. Additional methods specific to an experiment are described with the proposed experiment.

Subjects

All subjects were approved for participation in this study by a physician. All tests performed on the subjects received approval of Simon Fraser University's Human Subjects Ethics Approval Committee.

Ergometry

The cycle ergometer (Lode: Groningen, Holland) was electromagnetically braked and externally controlled by a PC compatible Megacom computer incorporated with an A/D, D/A interface (KEM Industries). A turbo C program was written to accept and control a variety of work rate protocols.

Cardiorespiratory data collection

Real time breath-by-breath $\dot{V}O_2$ acquisition was made using a Mac IIfx computer equipped with a National Instruments A/D board (NB-MIO-16) and a National Instruments software package (LabVIEW II). Expiratory timing (sail switch), expiration flow rate (modified from an Alpha Technologies Ventilation Module, model VMM110), F_EO_2 (Applied Electrochemistry, model S-3A), and F_ECO_2 (Applied Electrochemistry, model CD-3A), were recorded at 112 Hz (each channel) for each breath. T_T and T_E were recorded from the binary signal of the expiration sail switch. In addition, inspired volume (Alpha Technologies turbine), heart rate (Physio-Control monitor), work rate, and rpm were recorded each breath.

The flow rate, $F_{E}O_2$, and $F_{E}CO_2$ signals were first converted from a voltage to $l \cdot min^{-1}$, $\%O_2$, and $\%CO_2$, respectively. The $F_{E}O_2$ signal was inverted so that the baseline represented 0%. The flow rate, $F_{E}O_2$, and $F_{E}CO_2$ signals were then time aligned and compensated for the dynamic response of their respective recording instruments (Noguchi *et al.*, 1982). FRC was determined by the method of (Linnarsson, 1974). V_D (l) was determined by the calculation of Young (1955). V_A (l) was determined by subtracting V_D (l) from the integrated expired flow rate (V_E (l)). $\dot{V}EO_2$ ($l \cdot min^{-1}$) was calculated by multiplying the inverted $F_{E}O_2$ (%) signal by flow rate ($l \cdot min^{-1}$). Since $F_{E}O_2$ was inverted, the baseline which represents $F_{I}O_2$ was zero. However, there was a small $\dot{V}IO_2$ because the initial portion of each inspired breath contains expired air from the previous breath that remains in the breathing valve (90 ml). $\dot{V}IO_2$ was calculated by the method of Beaver *et al.* (1973). The change in O_2 stores within the lung FRC, due both to changes in volume and gas concentration in successive breaths, was determined from Linnarsson (1974). $\dot{V}O_2$ ($l \cdot min^{-1}$) was calculated by the equation:

$$\dot{V}O_2 = \dot{V}EO_2 - \dot{V}IO_2 \pm O_2 \text{ stores} \quad (7)$$

$\dot{V}CO_2$ was calculated similarily each breath.

The data saved each breath included: run time (min), $\dot{V}O_2$ ($l \cdot min^{-1}$: STPD), $\dot{V}CO_2$ ($l \cdot min^{-1}$: STPD), $\dot{V}E$ ($l \cdot min^{-1}$: BTPS), FRC (l: BTPS), $F_{ET}O_2$ (%), $F_{ET}CO_2$ (%), T_E (ms), T_T (ms), V_A (l: BTPS), V_I (l: BTPS), HR ($beats \cdot min^{-1}$), work rate (Watts), and pedaling frequency (rpm).

Data Analysis

Extraneous cardiorespiratory data, due to swallowing, coughing, line spikes, etc., were removed prior to data analysis.

Breath-by-Breath Verification

To verify the validity of the breath-by-breath $\dot{V}O_2$ data collection program, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$ values from the program were compared to values determined by the Douglas bag method

(Fig. 3-1, 3-2, and 3-3). The data for each method were acquired simultaneoulsy at steady state work rates ranging from 0 W to 250 W. Each breath-by-breath value represents an average of the last 20 breaths from 4 min of steady state exercise. The expired air from these 20 breaths was collected in a meterological balloon for subsequent determination of $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$. The figures indicate that the breath-by-breath real time calculation of $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$ were very comparable with corresponding values determined from bag calculations of concurrently collected data.

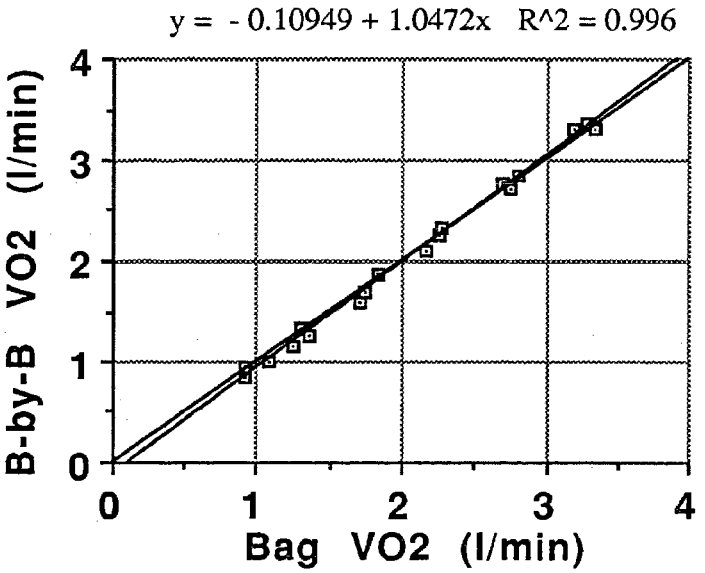

Fig. 3-1. A comparison of breath-by-breath (B-by-B) $\dot{V}O_2$ values with $\dot{V}O_2$ values determined from the Douglas bag method. The line through the origin represents the line of identity and the other line represents the best fit line through the data. The equation for the regression line is above the graph.

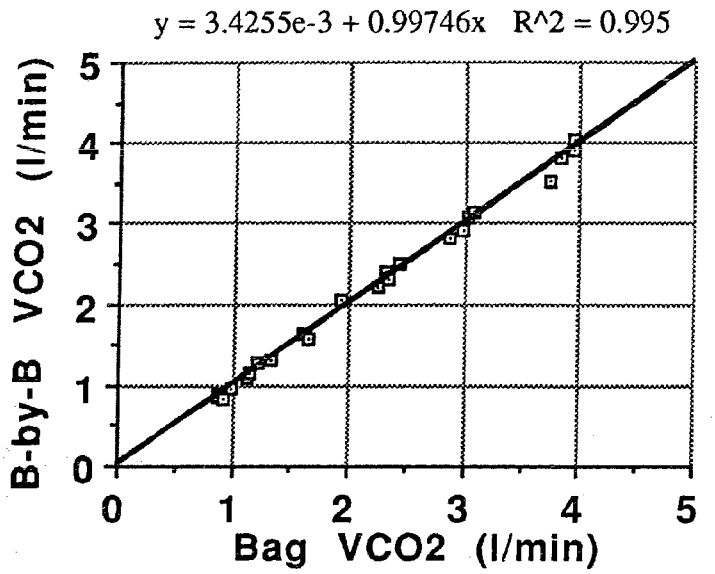

Fig. 3-2. A comparison of breath-by-breath (B-by-B) $\dot{V}CO_2$ values with $\dot{V}CO_2$ values determined from the Douglas bag method. The line through the origin represents the line of identity and the other line represents the best fit line through the data. The equation for the regression line is above the graph.

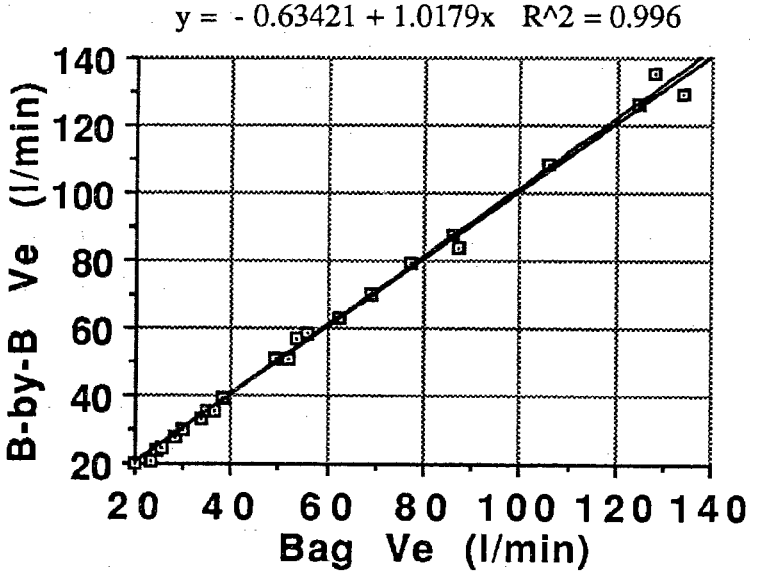

Fig. 3-3. A comparison of breath-by-breath (B-by-B) $\dot{V}E$ values with $\dot{V}E$ values determined from the Douglas bag method. The line through the origin represents the line of identity and the other line represents the best fit line through the data. The equation for the regression line is above the graph.

4.0 EXPERIMENT 1-SIGNAL PROCESSING SIMULATION

Introduction

The length of data selected to fit a single exponential is often recommended to be 4-6 time constants in duration. For example, if the $\dot{V}O_2$ time constant is 30 s then 120-180 s of data would be selected starting at the onset of the stimulus such as a step change in work rate. Lamarra *et al.* (1987) suggest a shorter duration of 3 time constants "since data lying near the steady state reduce the relative sensitivity of the parameter estimation within the transient region" (Lamarra *et al.*, 1987: p 2008). However, these authors do not present any empirical, theoretical, or simulation analysis to support this contention.

Techniques such as ensembling, interpolating, and smoothing have been used prior to curve fitting to quantify $\dot{V}O_2$ kinetic data better. Certainly, the superposition or ensemble averaging of many repetitions increases the accuracy of $\dot{V}O_2$ kinetic determination. Median values may represent ensembled data better than mean values, especially if the signal-to-noise ratio is low. Interpolation is often used to restructure $\dot{V}O_2$ data so that they occur at equal intervals of time. This allows parametric analysis of the data. Smoothing is used to increase the signal-to-noise ratio. However, the precise effects of all of the above data conditioning processes have not been quantified in relation to $\dot{V}O_2$ kinetic determination.

It is the purpose of this study to examine the effects of data length, median selection of ensembled data, interpolation, and smoothing on kinetic determination of simulated $\dot{V}O_2$ data. If a proposed curve fitting method proves superior to standard procedures by generating a more accurate time constant estimate or a lower standard deviation for the time constant estimate then fewer repetitions of data acquisition will be required. In any laboratory in which numerous samples are required to describe data better, such as standard curves in biochemistry or spike

triggered averaging in neurophysiology, any research tool which is faster and more reliable will save time and expense.

Methods

Simulated $\dot{V}O_2$ data were determined from a defined saturating exponential with a known time constant of 30 s and $\Delta\dot{V}O_2$ steady state of 0.914 $l \cdot min^{-1}$. Thirty seconds is a frequently reported time constant for an ON-step $\dot{V}O_2$ response. The simulated step increase was 100 W and the oxygen cost of 0.914 $l \cdot min^{-1}$ as determined by Lamarra *et al.* (1987) for the same work rate. Gaussian amplitude noise with a standard deviation of 0.091 was added to the underlying exponential. The standard deviation of 0.091 was the same as the median value of the 5 subjects used by Lamarra *et al.* (1987). Noise was also incorporated into the average breath interval of 3 s. The standard deviation of the breath interval noise was 0.33 s and thus the largest expected range for the breath interval would be 2-4 s.

Simulation one: the effect of sample length.

Five hundred $\dot{V}O_2$ arrays of approximately 120 s duration were generated. Each of these arrays was treated differently to examine the effects of sample length as follows:

1) for the first condition the array was not modified thus acting as a control;
2) for this treatment the simulated steady state value of 0.914 was added to the array starting at 121 s and continuing to 900 s;
3) for this condition the average value of the last 7 points (approximately 21 s) of the 120 s array was appended to the array from 121 s to 900 s;
4) for this treatment the curve fitted steady state value of the 120 s array was added to the array at 121 s to extend the array to 900 s.

Each of these 4 arrays was curve fitted iteratively with a single saturating exponential equation to estimate the time constant and steady state value.

Simulation two: median ensembling versus mean ensembling.

Five hundred sets of $\dot{V}O_2$ arrays approximately 120 s long were generated. Each set consisted of 3, 5, or 7 arrays, thus making a total of 1500, 2500, and 3500 arrays, respectively. The time constant and steady state value were determined by iterative curve fitting. For each set of 3, 5, or 7 arrays, the time constant and steady state value were determined in 4 different ways:

1) the median time constant and steady state value of each 3, 5, or 7 individual arrays of the set;
2) the mean time constant and steady state value of each 3, 5, or 7 individual arrays of the set;
3) the time constant and steady state value of a single curve generated by ensembling the 3, 5, or 7 arrays and selecting the median value to construct the single ensembled curve;
4) the time constant and steady state value of a single curve generated by ensembling the 3, 5, or 7 arrays and selecting the mean value to construct the single ensembled curve.

For conditions 3 and 4 the arrays were interpolated to values every second prior to ensembling.

Simulation three: smoothing and interpolation effects.

Five hundred $\dot{V}O_2$ arrays approximately 120 s long were generated and each was processed by 5 different treatments as follows:

1) nothing was done to the array thus acting as a control;
2) each array was smoothed with a 5 point moving average;
3) each array was interpolated every second;
4) each array was interpolated every second and then smoothed;
5) each array was smoothed and then interpolated every second.

The control array and the 4 conditioned arrays which resulted from manipulating the control array were each curve fitted iteratively to estimate the time constant and steady state value.

Each of the above simulations generated 4 or 5 series of time constants in which N=500. The time constants for each condition of each simulation were compared to the theoretical perfect curve fit of 30 s with a Student's correlated *t*-test. In order to comprehend the magnitude of the data conditioning effects better, frequency tables were generated based on whether the estimated time constant of a procedure was better or worse than another procedure within 0.1, 1, 2, 3 s etc. The Chi Square test was used to determine significance for frequency analysis.

All modeling, data processing, and curve fitting were performed on a Macintosh IIfx computer (Apple Computer) using LabVIEW software (version 2.1.1: National Instruments). Statistical analysis was done with Microsoft Excel (version 2.2) on the Macintosh IIfx.

Results

The effect of lengthening sample duration with a constant had a variable effect on the time constant estimate (Tables 4-1 and 4-4). Extending the data with the known steady state value (0.914 $l \cdot min^{-1}$) did not give a better average estimate than the control condition (Table 4-1) but did produce more frequent results closer to the true underlying time constant of 30 s (Table 4-4) due to the lower standard deviation of the time constant estimate from data extension (Table 4-1). Extending the data with the mean of the last 7 points of the 120 s sample produced a poorer estimate of the underlying time constant (Tables 4-1 and 4-4). If the data were extended with the curve-fitted estimate, there was no change in the ability to accurately determine the true time constant (Tables 4-1 and 4-4).

TABLE 4-1. *Simulation one· Mean tau (s) and steady state ($l \cdot min^{-1}$) values for each data lengthening procedure.*

	Control	Extended 0.914	Extended Mean last 7 points	Extended Steady state of normal
Tau	30.27	30.08	28.15*	30.21
S.D.	3.36	1.81	2.98	3.29
Steady state	0.916	0.915	0.891	0.917
S.D.	0.032	0.001	0.032	0.032

* significantly different ($p < 0.01$: two-tailed t-test) from 30 s.

S.D. is the standard deviation.

TABLE 4-2. *Simulation two. Mean tau (s) and steady state (SS) ($l \cdot min^{-1}$) values for each group of 3, 5, and 7 arrays.*

Arrays per group	Three		Five		Seven	
	Tau S.D.	SS S.D.	Tau S.D.	SS S.D.	Tau S.D.	SS S.D.
Median of individual arrays	29.94 2.17	0.913 0.021	30.17 1.86	0.916 0.017	29.93 1.52	0.914 0.014
Average of individual arrays	29.98 1.85	0.906 0.018	30.27* 1.56	0.917 0.014	30.15* 1.23	0.916 0.011
Ensemble median	29.38* 2.31	0.906 0.024	29.52* 1.87	0.907 0.021	29.41* 1.51	0.906 0.018
Ensemble average	28.87* 2.12	0.900 0.024	28.90* 1.87	0.899 0.021	28.70* 1.51	0.898 0.018

* significantly different ($p < 0.01$: two-tailed t-test) from 30 s.

S.D. is the standard deviation.

The effect of using the median or mean to process data was dependent on the exact process used. For individual arrays, the median procedure gave a better average estimate of the underlying 30 s time constant for sets of 5 and 7 arrays (Table 4-2) yet the mean procedure produced more values closer to 30 s (Table 4-5) due to its lower standard deviation (Table 4-2). Ensembling the individual arrays prior to time constant estimation produced average estimates which were significantly smaller than the underlying true time constant value (Table 4-2). Using median values to ensemble the data was somewhat better than using mean values for ensembling (Table 4-5).

TABLE 4-3. *Simulation three. Mean tau (s) and steady state ($l \cdot min^{-1}$) values for each data conditioning procedure.*

	Control	5 point smooth	Interpolate	Interpolate then smooth	Smooth then interpolate
Tau	30.28	30.73*	30.31#	30.37#	30.73*
S.D.	3.45	3.41	3.48	3.48	3.42
Steady state	0.917	0.919	0.918	0.918	0.919
S.D.	0.032	0.032	0.033	0.033	0.032

* significantly different ($p < 0.01$: two-tailed t-test) from 30 s.

significantly different ($p < 0.05$: two-tailed t-test) from 30 s.

S.D. is the standard deviation.

TABLE 4-4. *Simulation one. Frequency count of how often a group was better than the control group by a given range of time.*

Range	≥ 0.1 s	≥ 0.5 s	≥ 1.0 s	≥ 2.0 s	≥ 3.0 s	≥ 5.0 s	≥ 7.0 s	≥ 9.0 s
0.914 better	337	291	248	157	88	24	6	1*
Control better	144	104	61	19	1	0	0	0
7 points better	212	179	145	90	57	18	2	0
Control better	276	239	204	137	69	5	0	0*
SS better	95	5	3	1	0	0	0	0
Control better	83	0	0	0	0	0	0	0

* significantly better ($p < 0.01$: two-tailed Chi squared test) than compared group.

Smoothing, interpolation, or the combination of the two in either order resulted in time constants that were significantly greater than the true 30 s value (Table 4-3). Since the average estimate was still within 0.5 s of the control value and there was no change in the standard deviation, the comparison of the individual time constant estimates for each processing condition used with the control condition produced no significant effects.

TABLE 4-5. *Simulation two. Frequency count of how often a group was better than another group by a given range of time (0.1 to 3 s).*

	Three arrays				Five arrays				Seven arrays			
Range (s)	≥0.1	≥1.0	≥2.0	≥3.0	≥0.1	≥1.0	≥2.0	≥3.0	≥0.1	≥1.0	≥2.0	≥3.0
Median idv better	192	34	8	2	190	33	0	0	158	31	1	0
Average idv better	261	99	34	2*	265	86	9	1*	274	78	9	0*
Ensemble median better	172	41	8	0	190	34	10	1	167	22	0	0
Average idv better	297	128	40	24*	270	98	42	24*	275	79	40	24*
Ensemble average better	156	45	11	5	168	54	16	5	150	51	8	0
Average idv better	299	135	62	28*	291	141	73	25*	316	175	80	22*
Ensemble average better	214	80	22	6	216	74	14	1	192	48	8	0
Ensemble median better	251	90	16	1*	253	102	23	5	285	106	20	2*
Ensemble median better	215	92	29	7	238	87	22	8	228	89	12	2
Median idv better	254	138	51	23*	237	113	46	21	237	94	38	21*
Ensemble average better	207	100	37	11	205	92	29	11	189	75	19	4
Median idv better	273	150	73	28*	273	150	63	23*	186	164	71	24*

* significantly better ($p < 0.01$: two-tailed Chi squared test) than compared group.

idv = individual.

TABLE 4-6. *Simulation three. Frequency count of how often a group was better than the control group by a given range of time (0.1 to 2 s).*

Range	≥ 0.1 s	≥ 0.5 s	≥ 1.0 s	≥ 2.0 s
Smooth better	243	119	25	0
Control better	209	126	38	0
Interpolate better	194	77	17	0
Control better	237	92	18	1
Interpolate-Smooth better	204	81	15	0
Control better	236	100	18	1
Smooth-Interpolate better	249	124	32	0
Control better	207	134	47	3

Discussion

Simulation one showed that artificially extending the steady state duration of an exponentially saturating array will both improve the estimate of the time constant (Table 4-4) and reduce the deviation of the estimate (Table 4-1). However, this was dependent on the manner in which the data were extended. Extending the data by using the mean of the last 7 points or using the estimated steady state value for the 120 s array did not improve the fitting procedure. This may be rectified if the duration of the simulated data were lengthened past 120 s thus enabling a better estimate of the actual steady state value. The better estimate of tau attained when extending the data with the true steady state value is contrary to the intuitive statement by Lamarra *et al.* (1987) that a longer sample time reduces the sensitivity of the tau estimate. Although I do not have a mathematical proof of this effect, it may be due to forcing the nonsteady state section of the curve towards the underlying true curve because the steady state response is constrained to its true value.

The effect of ensembling data, as done in simulation two, appears to be negative. Whether 3, 5, or 7 arrays were ensembled, the estimated time constant was significantly different from 30 s irrespective of whether the data were ensembled by median or mean functions (Table 4-2). Ensembling with a median function did generate a better time constant estimate than using a mean function. Such an effect may be even more pronounced if the data included some bad values as is likely to happen during real $\dot{V}O_2$ data collection due to a subject swallowing or coughing.

Rather than ensembling the data, taking a median or mean estimate of the individual time constant was generally better (Tables 4-2 and 4-5). However, the results were conflicting about whether it is better to take the median or mean value of the individually fitted arrays. The median function provided a better estimate of the time constant according to the data presented in Table 4-2, whereas the frequency counts in Table 4-4 indicated that the mean function is superior. This apparent contradiction can be explained. The mean function altered the true value of the time constant but at the same time reduced the standard deviation of the time constant estimate. Thus, while the mean of the distribution may be shifted somewhat, the smaller standard deviation resulted in a greater frequency of values closer to 30 s.

The well known effect of increasing the number of arrays ensembled to increase the signal to noise ratio was very evident by the reduction of the standard deviation as the number of arrays ensembled increased (Table 4-2). This effect was also apparent in the reduction of values in the range of 2 to 3 s as the number of arrays ensembled increased (Table 4-5).

Smoothing and interpolation are often used to condition data. Smoothing is used to increase the signal-to-noise ratio. This usually results in a greater visual clarity of any apparent trends in the data although the waveform is modified somewhat. Interpolation is used as a prelude to ensembling so that points to be ensembled are equally distributed in time. Interpolation also enables parametric analysis to be done because the data are converted to an evenly time distributed state. However, smoothing, and to a smaller extent interpolation, both reduced the ability to estimate the time constant accurately (Table 4-3). There was very little consistent difference in the

frequency counts (Table 4-6). This was likely due to the unaltered standard deviations. There was no effect of the ordering of interpolating and smoothing when compared to control condition.

In summary, the most valid and reliable way to estimate a time constant from non-linear least squares curve-fitting of array data to single exponential form is to determine the steady state accurately and use this value to artificially extend the data (30 time constant durations in simulation one) prior to curve fitting. Other data conditioning processes such as ensembling, smoothing, and interpolation may improve visual presentation, but do not improve time constant estimation.

5.0 THE RELATION OF $\dot{V}O_2$ WITH WORK RATE DURING A RAMP WORK RATE PROTOCOL

5.1 REVIEW OF LITERATURE

It is widely accepted that at work rates above the lactate threshold, $\dot{V}O_2$ kinetics exhibit a more complex behaviour than can be described by a linear first order system. There is some controversy however, whether a similar conclusion may be applied to the $\dot{V}O_2$ response when the work rate is held below the work rate equivalent to the lactate threshold. Certainly, the kinetic behaviour of the $\dot{V}O_2$ response is influenced by several factors including: muscle oxygen uptake ($\dot{Q}O_2$), the change in the O_2 stores (pulmonary, venous, and muscular sources), the temporal delay of the $\dot{V}O_2$ signal arriving at the lungs from the muscle, and the change in cardiac output during the time it takes the $\dot{V}O_2$ response to travel from the muscle to the lungs (Whipp and Ward, 1990).

Linearity of the $\dot{V}O_2$ response implies that the ON $\dot{V}O_2$ kinetics should be the same for different step increases in work rate. Additionally, the ON $\dot{V}O_2$ response should be the same as the OFF $\dot{V}O_2$ response. However, $\dot{V}O_2$ kinetics encompassing the O_2 debt appear more complex and less well understood than the $\dot{V}O_2$ kinetics describing the O_2 deficit (Bangsbo *et al.*, 1990). For this reason, studies that have investigated the OFF $\dot{V}O_2$ response, with respect to the linearity of the $\dot{V}O_2$ response, will not be reviewed.

Henry (1951) indicated that the amplitude of the increase in $\dot{V}O_2$ above a baseline value ($\Delta\dot{V}O_2(ss)$) in response to a step increase in work rate and the time constant of the response ($\dot{V}O_2$ tau) are mathematically independent and thus the $\dot{V}O_2$ time constant will be independent of the amplitude of the step change in work rate. If $\dot{V}O_2$ kinetics respond as a first-order system then the phase 2 (see Fig. 2-3 and 2-3) $\dot{V}O_2$ time constant for a step increase in WR should be the same for all steps below lactate threshold, and perhaps even to step increases above the lactate threshold. The studies listed in Table 5-1 have investigated ON $\dot{V}O_2$ kinetics. The general trend of these data

indicates that tau $\dot{V}O_2$ increases with increasing work rate. However, due to inconsistencies in determining the phase 2 $\dot{V}O_2$ time constant in these experiments, a conclusive statement cannot be made about the linearity of $\dot{V}O_2$ kinetics for a step increase in work rate from a baseline of rest or unloaded pedaling in cycle ergometry. Barstow and Mole (1991) did specifically determine the phase 2 $\dot{V}O_2$ time constant for 4 different step changes in work rate (2 below the work rate corresponding to the lactate threshold and 2 above). They found the phase 2 time constant to be invariable with work rate. However, their data did indicate a trend for an increasing time constant. Perhaps with more than 4 repetitions at each work rate and more than 4 subjects, the trend of an increasing time constant would be statistically significant.The linearity of the $\dot{V}O_2$ response may also be determined from ramp data. The $\dot{V}O_2$ response to a ramp work rate protocol is the same regardless of the baseline work rate indicating a degree of linearity (Yoshida, 1990). The $\dot{V}O_2$ response time should also be the same for different ramp slopes. While some studies have demonstrated a similar response time for different ramp slopes (Davis *et al.*, 1982; Yoshida, 1990) other studies have not (Swanson and Hughson, 1988). Like the step WR protocol, the linearity of the $\dot{V}O_2$ response to ramp work may be confounded at work rates above the lactate threshold (Hansen *et al.*, 1988).

Several investigators (Davis *et al.*, 1982; Hughson and Inman, 1986b; Murphy *et al.*, 1989; Swanson and Hughson, 1988; Whipp, 1987) have determined the $\dot{V}O_2$ time constant from ramp data as shown in Fig. 2-6. Using this method they have assigned a single $\dot{V}O_2$ time constant to fit all data points. This implies that a single time constant describes the time course for all work rates (Fig. 5-1). Even if the $\dot{V}O_2$ response is linear throughout the ramp protocol, more than one time constant could contribute to the $\dot{V}O_2$ response and not be detected, if the additional component with a different time constant was invoked at the same time.

TABLE 5-1. *Oxygen uptake time constants[a] (s) for leg cycling ergometry determined from step work rate changes.*

Reference						
Whipp, 1971	R-300 kg-m/min[b]	R-450				
	44.4	44.4				
Whipp and Wasserman, 1972[c]	R-50 W	R-75	R-100			
	39	48	56			
Whipp et al. 1982	R-100 W	0-100				
	28.6	28.9				
Sietsema et al., 1989	MR-25 W	MR-50	MR-100	MR-150		
	14	22	37	47		
Linnarsson, 1974	?-80	?-160	?-240			
	38.7	40.6	45.6			
Hagberg et al., 1978[c]	R-300 kp-m/min	R-600	R-900	R-1050	R-1200	R-1500
Untrained	17	29	35	40	48	43
Trained	20	19	26	32	29	40
Cerretelli et al., 1977[c]	R-0.8 l/min $\dot{V}O_2$	R-1.3	R-1.8	R-2.5		
Supine	63	71	84	79		
Upright	--	52	--	62		
DiPrampero et al., 1989[c]	R-20 %$\dot{V}O_2$ max	R-40	20-40	25-85		
	23.2	43.1	51.9	70.7		
Bason et al., 1973[c]	R-30 %$\dot{V}O_2$ max	R-60				
	61	94				
Hughson et al., 1988	25-65 W	65-105	25-105	25-145		
	19.1	26.7	23.0	23.7		
Hughson and Morrissey, 1982	R-40% LT	R-40% LT	R-80% LT	40-80% LT		
	30.0	39.6	37.8	60.6		
Zhang et al., 1991[d]	0-25 %$\dot{V}O_2$ max	25-50 %	50-75 %	75-100 %		
	38.3	45.7	57.2	68.0		
Casaburi et al., 1989a	0-25 W	0-51	0-76	0-101	0-127	0-152
	32.3	34.6	38.1	44.4	52.3	68.9

LT = Lactate threshold
MR = Moving rest. A motor moves the pedals at rest.
R = rest.
? = baseline conditions not mentioned.
[a] When more than 1 exponential equation was curve fitted, the time constant corresponding to phase 2 is indicated.
[b] The step work rate protocol is given in first data column only.
[c] Time constants were converted from time to reach 50 % of steady state.
[d] Time constants were converted from time to reach 75 % of steady state.

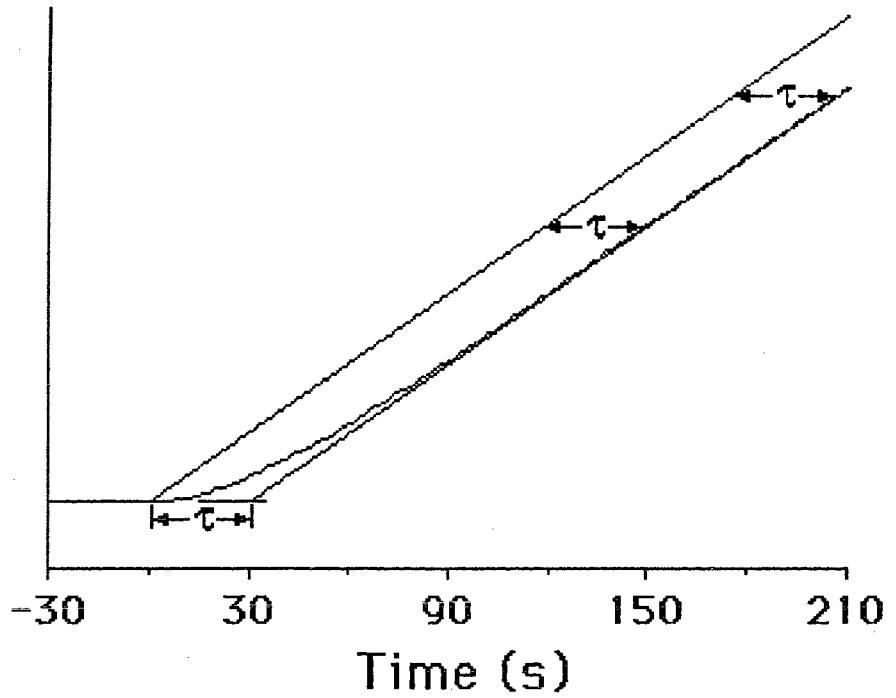

Fig. 5-1. $\dot{V}O_2$ response to a ramp work rate protocol as in Fig. 2-5 and 2-6. It can be observed that the time constant (τ) in this conventional representation is the same no matter at what point of the $\dot{V}O_2$ time course it is determined.

If the $\dot{V}O_2$ response to the ramp work rate demand progressively diverges from the work rate, rather than paralleling it, then rate of aerobic ATP production progressively diverges from rate of ATP production demanded by the work rate. This implies that the anaerobic ATP production rate increases with work rate (see Section 2 Fig. 2-7). Since the anaerobic ATP contribution is progressively increasing, by default the $\dot{V}O_2$ time constant must become progressively longer (Fig. 5-2).

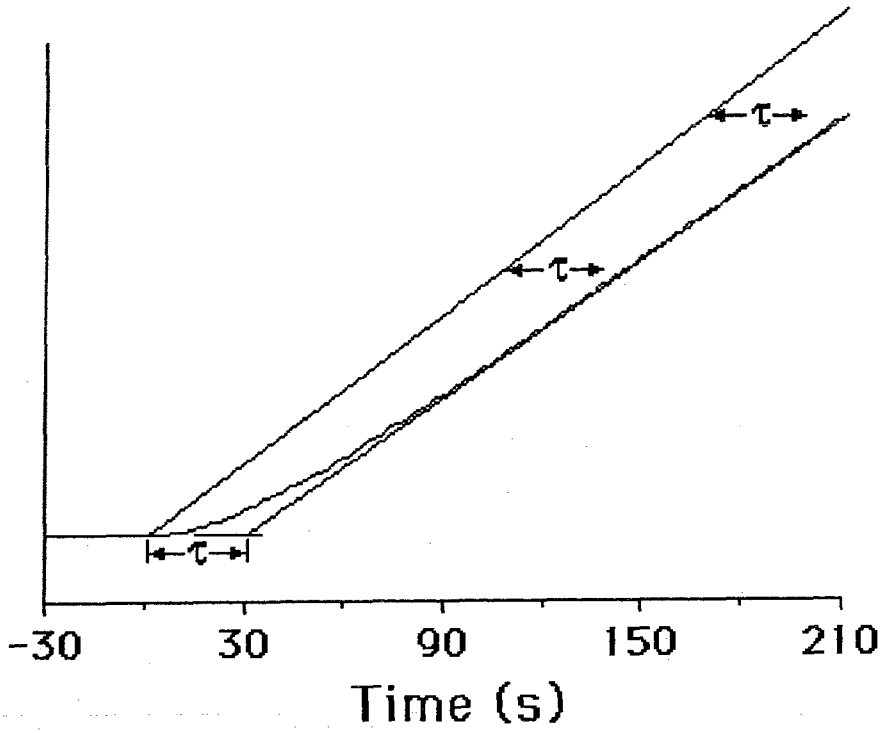

Fig. 5-2. $\dot{V}O_2$ response to a ramp work rate protocol as in Fig. 2-7. It can be seen that the time constant (τ) increases as work rate increases because the slope of the observed $\dot{V}O_2$ response is less than the slope of the rate of ATP production demanded by the ramp work rate.

It is difficult to allocate components of the aerobic and anaerobic response precisely to specific points along the work rate forcing function, from whole body studies. Accurate quantification of the anaerobic and aerobic energy contribution to ATP production has yet to be accomplished. Thus it is not possible to present $\dot{V}O_2$ ATP provision and work rate ATP demand in their precise relative position graphically. However, it is still possible to determine if ramp $\dot{V}O_2$ data diverge from, or parallel, the ramp work rate ATP demand. Some evidence for a $\dot{V}O_2$ slope divergence from work rate during an incremental ramp work rate has been presented by Murphy *et al.* (1989). These researchers observed the $\dot{V}O_2$ response to a 40 $W \cdot min^{-1}$ ramp under normoxic and hypoxic conditions. A schematic figure based on their data indicates that the slope of the hypoxic response is less than the slope of the normoxic response (Fig. 5-3).

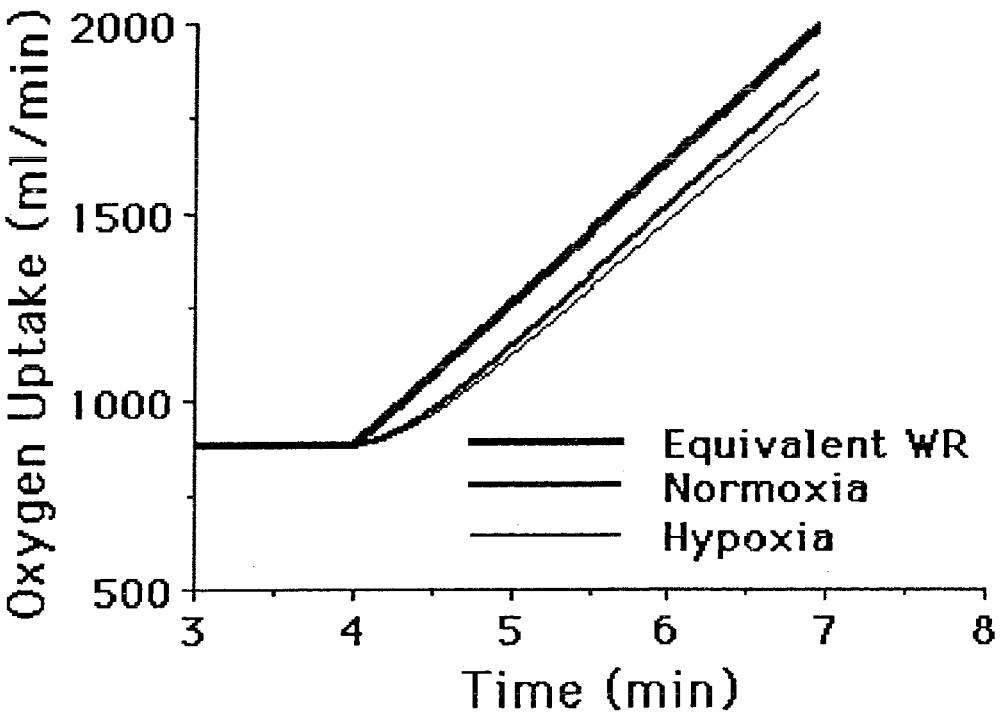

Fig. 5-3. A schematic composition of data from Murphy *et al.* (1989). The normoxic $\dot{V}O_2$ response was plotted from a slope of 9.4 ml $O_2 \cdot min^{-1} \cdot W^{-1}$ and a total lag time (time constant + time delay) of 19.7 s. The hypoxic curve was plotted from a slope of 9.0 $ml \cdot min^{-1} \cdot W^{-1}$ and a total lag time of 21.4 s. The equivalent work rate was derived from the normoxic curve using a time constant of 0.001 s and no lag time.

Since the work rate ramp was the same for both the hypoxic and normoxic protocols it is apparent that the hypoxic $\dot{V}O_2$ response progressively diverges more from the work rate induced ATP demand than does the normoxic $\dot{V}O_2$ response curve. Predictably, a hyperoxic condition may in turn demonstrate a steeper $\dot{V}O_2$ slope than the normoxic condition. This would be strong evidence that the normoxic response also diverges from the work rate induced ATP demand.

Whether or not $\dot{V}O_2$ kinetics exhibit a first order response to a work perturbation can also be determined by using different types of work rate stimuli. The ability to predict a step $\dot{V}O_2$ response based on data collected from an impulse work rate is based on the 'principle of superposition'.

Examination of the literature for studies that have recorded $\dot{V}O_2$ kinetic data using different exercise stimuli are, like ON step data, inconclusive. Whipp *et al.* (1981) found that the kinetic parameters determined from a step change in work rate were very similar to those determined from a ramp change in work rate. In contrast, Hughson and co-workers demonstrated that the $\dot{V}O_2$ kinetic parameters of an impulse function cannot be used to describe the time course of a step function (Hughson *et al.*, 1988). Similarly, $\dot{V}O_2$ kinetic parameters of a step function cannot be used to describe ramp work rate $\dot{V}O_2$ kinetics (Swanson and Hughson, 1988). Thus, the $\dot{V}O_2$ response appears nonlinear. However, such comparisons still remain equivocal since the latter investigation used an inappropriate model to describe the observed results. For the $\dot{V}O_2$ response to a step work rate, a parallel 2 component step model was used; one component was attributed to phase 1 and the other component to phase 2. Swanson and Hughson (1988) suggest that the phase 1 compartment of the model reflects the change in blood flow and distribution and the phase 2 compartment represents O_2 extraction by the working muscles. It seems more logical to assume that the phase 1 response is a transient function better modeled as an impulse response, not with a step function which lasts the duration of the work rate step. The phase 1 cardiorespiratory response lasts 10-20 s and may be attributed to a 'surge' of venous blood to the cardiopulmonary system (Casaburi *et al.*, 1989b). Regardless of the cause, the effect is considered transitory (Casaburi *et al.*, 1989b). Some investigators have found that the phase 1 response during a step to a light work rate may actually be larger than the phase 3 response (Sietsema *et al.*, 1989). Thus fitting phase 1 with a step function appears inappropriate, leading to erroneous estimation of phase 2 kinetic parameters.

$\dot{V}O_2$ kinetics may indeed be a first-order linear system and the stated uncertainty about linearity indicated by some investigators may lie more in the methodology used to analyse the system than in the fact of a real difference in kinetics. Analysis of whole body metabolism from breath-by-breath O_2 utilisation may be too insensitive to detect a linear response at the working muscle.

When the $\dot{V}O_2$ response is determined directly from an isolated muscle preparation, $\dot{V}O_2$ linearity is more prominent although few studies have been conducted.

The time course of muscle O_2 utilisation ($\dot{Q}O_2$) in an *in situ* dog preparation was examined when the $\dot{Q}O_2$ was increased between 4-15 fold above rest (DIPrampero and Margaria, 1968). Although there was only one repetition per step increase in $\dot{Q}O_2$ per dog, the authors reported that the different $\dot{Q}O_2$ steps were all described well by a single exponential with the same time constant. This is consistent with a first order linear model. Mahler, provided strong support for the linearity of the $\dot{V}O_2$ response from experiments with an isolated frog sartorius muscle preparation. Mahler noted the single exponential behaviour of the $\dot{V}O_2$ response after an impulse work rate stimulation (Mahler, 1978). The robust nature of the response was verified when it was observed that heat production followed a similar time course (Mahler, 1980).

5.2 EXPERIMENT 2- FIO_2 INFLUENCES ON $\dot{V}O_2$ DURING RAMP WORK

Introduction

The time course of the observed $\dot{V}O_2$ above a baseline induced by ramp incremental work rate is considered to parallel the steady state demand after a time delay of four to five $\dot{V}O_2$ time constants (Fig. 5-1). This is implicit in the mathematics conventionally used to describe the $\dot{V}O_2$ response to a work rate of this type.

$$\Delta\dot{V}O_2(t) = \Delta\dot{V}O_2(ss) \cdot [t - \tau \cdot (1 - e^{-t/\tau})] \quad (5\text{-}1)$$

When $t \gg \tau$, $\Delta\dot{V}O_2(t)$ lags $\Delta\dot{V}O_2(ss)$ by the constant τ. However, it has been observed that the slope of the $\dot{V}O_2$ response is less during hypoxia than during normoxia (Fig. 5-3) (Murphy *et al.*, 1989). This is good evidence that at least the hypoxic $\dot{V}O_2$ response diverges from parallelism with the incremental metabolic demand since the work rate protocol was the same for both conditions. However, it is not known whether or not the normoxic $\dot{V}O_2$ response also diverges

from metabolic demand. A possible way to determine this relation is to compare the normoxic $\dot{V}O_2$ response with the hyperoxic $\dot{V}O_2$ response. By making the assumption that $\dot{V}O_2$ will not exceed metabolic demand, if the hyperoxic $\dot{V}O_2$ response-WR relation has a greater slope than the normoxic $\Delta\dot{V}O_2/\Delta WR$ relation, then the normoxic $\dot{V}O_2$ response will be considered to have diverged from the metabolic demand of the imposed work rate.

It is the purpose of this experiment to determine the slope of the $\dot{V}O_2$ response to the same ramp work rate protocol in hyperoxia, normoxia, and hypoxia.

Methods

The subjects were all healthy males and experienced in $\dot{V}O_2$ cycle ergometry. A physical examination was conducted by a physician prior to participation in the study. Their physical characteristics are listed in Table 5-2.

The FIO_2 conditions consisted of hypoxia (12% O_2, balance N_2), normoxia (21% O_2, balance N_2), and hyperoxia (40% O_2, balance N_2). For each condition, the source of the inspired air was a compressed gas cylinder. The air was bubbled through 10 liters of water in 200 litre meteorological balloon before it was inspired.

The work rate protocol consisted of 3 phases: 10 min of rest then 8 min at 30 W followed by a 1 $W \cdot 3\ s^{-1}$ (20 $W \cdot min^{-1}$) ramp work rate until exhaustion. The selected inspired gas was used for all 3 phases. Each subject performed 2 rides for each FIO_2 condition.

Data Analysis

Extraneous data (usually with TT less than 600 ms), due to swallowing etc. were removed prior to analysis. The portion of the $\dot{V}O_2$ response used for slope determination was started 3 min after ramp work rate onset and ended 1 min prior to exhaustion. The anaerobic threshold was estimated from respiratory gas exchange parameters and thus will be referred to as the respiratory gas exchange threshold (RGET). The linear slope of the $\dot{V}O_2$ response for the whole ride, the

portion prior to the RGET (pre RGET), and the portion subsequent to the RGET (post RGET) was determined. The RGET was determined by the V-slope method of Beaver *et al.* (1986). In certain instances, more so for the hypoxic condition, relative hyperventilation made it difficult to determine the RGET. In such cases other indicators such as the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$ were used in aiding the RGET determination. The precise point of the RGET was determined using the algorithm of Veith (1989). All data were smoothed with a 5 point moving average prior to RGET determination.

Statistical analysis

A 3-way ANOVA with repeated measures was done with two slope factors (pre and post RGET $\Delta\dot{V}O_2/\Delta WR$ slopes), three FIO_2 conditions (hypoxia, normoxia, and hyperoxia) and two time conditions (test 1 and test 2). A 2-way ANOVA with repeated measures was done with the 3 FIO_2 conditions and two time conditions for each of the pre RGET, post RGET, and whole $\Delta\dot{V}O_2/\Delta WR$ slopes. Pre and post $\Delta\dot{V}O_2/\Delta WR$ slopes were analyzed with a 2-way ANOVA with repeated measures (2 levels of slope and 2 levels of time). RGET work rates, maximal work rates, and 30 W steady state and maximum values for $\dot{V}O_2$, $\dot{V}E$, and HR were analyzed with a 2-way ANOVA with repeated measures with 3 FIO_2 factors and 2 time factors. The percent RGET work rates were analyzed with a 2-way ANOVA with repeated measures (3 FIO_2 conditions and 2 time conditions). All preplanned individual comparisons were made with correlated t-test. Probabilities are two-tailed unless reported otherwise. Differences were considered significant at with $p \leq 0.05$.

Supplemental EMG experiment

The $\dot{V}O_2$ response to ramp exercise was found to be different for different FIO_2 conditions. To gain some insight into possible mechanisms of this effect, EMG was recorded on subject #1 for all 3 inspired gas conditions. The EMG electrodes were placed on the vastus medialis 17 mm apart (centre to centre). A preamplifier (Grass P-15) was used with a low cut-off frequency of 30 Hz

and a high cut-off frequency of 300 Hz. The EMG signal was sampled at 1000 Hz and converted to digital output with a PCL-812PG A/D converter (Advantech Co. Ltd., San Jose, Calif.). The sample period was 5 s. Samples were taken during the 30 W baseline work rate and every minute (20 W) during the ramp exercise. EMG data acquisition was controlled with LABTECH Notebook software on an IBM compatible (Zenith Z-286 LP Plus) computer. After the experiment, the data were transferred to a Macintosh IIfx computer for further processing. Programs were written with LabVIEW software to calculate the integrated EMG (IEMG), median power frequency (MedPF), mean power frequency (MeanPF), and zero crossings (ZC). At each work rate, the first 6 complete bursts, encompassing 4300 ms, were used in data analysis. Each burst represents the down stroke of the leg from which the EMG was measured. Although it is recommended to restrict power spectral analysis to stationary data (Hagg, 1992), this is for reasons of estimate stability and not validity. In addition, the work rate change during 4300 ms represents only about 0.5 % of the average work rate change for the present experiment.

TABLE 5-2. *Characteristics of subjects.*

Subject	Age	Height (cm)	Weight (kg)	$\dot{V}O_2max$ ($l \cdot min^{-1}$)
1	39	186	86	3.99
2	28	181	68	3.98
3	23	187	82	4.17
4	27	170	64	4.04
5	30	180	86	4.35
6	23	175	79	3.99
7	32	180	70	3.99

Supplemental ramp slope experiment

The effects of time and previous history of a recruited motor unit may influence its response to a work rate perturbation. With this in mind, subject #1 repeated the normoxic FIO_2 condition but with a step-negative ramp work rate protocol. The subject rested for 10 min, exercised at 30 W for 8 min, then the WR was increased to 330 W and decremented by 1 W·3 s^{-1} down to 30 W.

Results

The group average $\Delta\dot{V}O_2/\Delta WR$ relation are presented in Table 5-3 and Fig. 5-4. The overall effect of the FIO_2 condition on the $\Delta\dot{V}O_2/\Delta WR$ slope was significant ($F = 27.32$, $p < 0.0001$) and the interaction of gas and slope was also significant ($F = 6.42$, $p < 0.01$). For the pre RGET range of the $\Delta\dot{V}O_2/\Delta WR$ relation, the normoxic ($F = 31.32$, $p < 0.001$) and hyperoxic ($F = 23.15$, $p < 0.003$) slopes were significantly different from the hypoxic condition whereas the normoxic response was not different from the hyperoxic response ($F = 0.85$, $p = 0.39$) (Table 5-3). For the post RGET range of the $\Delta\dot{V}O_2/\Delta WR$ relation, hypoxia was significantly different from normoxia

TABLE 5-3. *The group average oxygen uptake versus work rate slope (ml $O_2 \cdot min^{-1} \cdot W^{-1}$) (± SD) for each inspired oxygen condition.*

	Pre RGET	Post RGET	Whole
Hypoxia	$9.34 \pm 0.89^{b,c}$	$8.41 \pm 0.97^{b,c,d}$	$8.91 \pm 0.63^{b,c}$
Normoxia	10.19 ± 1.04^{a}	$9.96 \pm 1.10^{a,c}$	$10.40 \pm 0.77^{a,c}$
Hyperoxia	10.44 ± 0.72^{a}	$11.56 \pm 0.78^{a,b,d}$	$11.08 \pm 0.48^{a,b}$

[a] significantly different from hypoxia.

[b] significantly different from normoxia.

[c] significantly different from hyperoxia.

[d] significantly different between pre and post RGET.

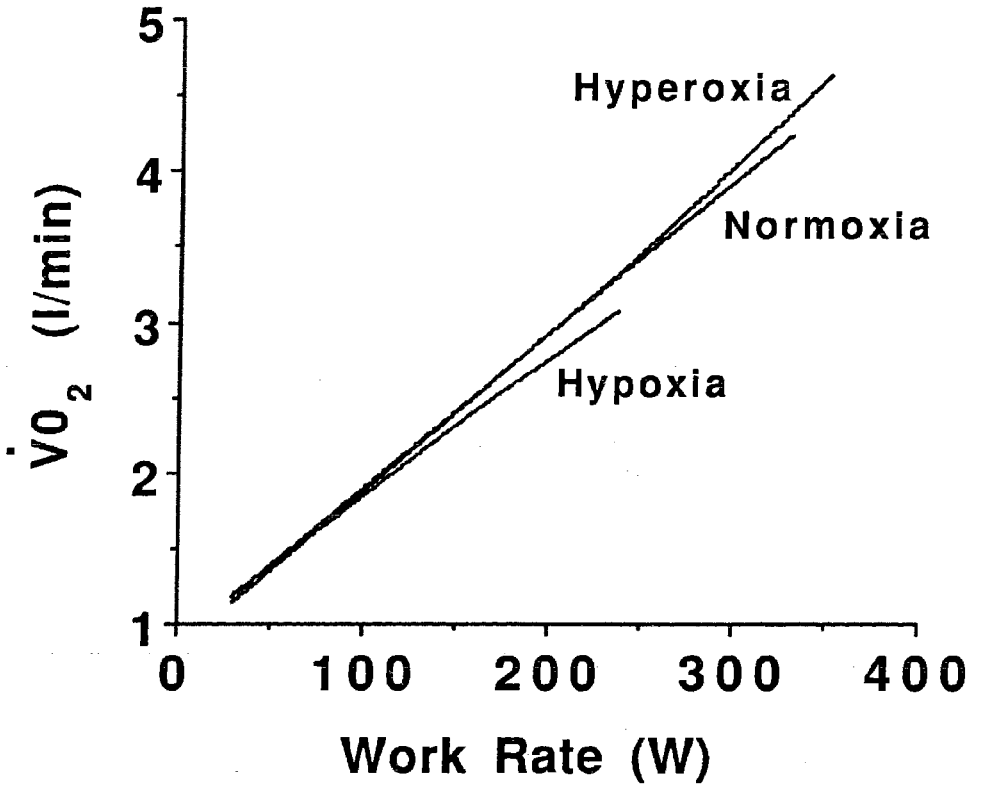

Fig. 5-4. The average $\dot{V}O_2$ response to work rate for each gas condition.

(F = 6.35, p = 0.045) and hyperoxia (F = 6.67, p = 0.04) (Table 5-3). For the whole $\Delta\dot{V}O_2/\Delta WR$ relation (3 min after start of ramp exercise until 1 min from exhaustion), hypoxia was significantly different from normoxia (F = 49.14, p = 0.0004) and hyperoxia (F = 139.7, p < 0.0001) and normoxia was significantly different from hyperoxia (F = 15.17, p = 0.008) (Table 5-3). In comparison to the pre RGET $\Delta\dot{V}O_2/\Delta WR$ range, the post RGET range was lower for hypoxia (F = 5.16, p = 0.032 one-tailed), not different for normoxia (F = 0.15, p = 0.72), and greater for hyperoxia (F = 7.23, p = 0.036) (Table 5-3).

RGET work rates and maximal work rates were, in general, reduced with decreasing FIO_2 (Fig. 5-5). The work rate equivalent to the RGET was different between gases (F = 155.61, p < 0.0001). Individual comparisons indicated that RGET WR for hypoxia was significantly less than that for normoxia (F = 184.32, p < 0.0001) and hyperoxia (F = 377.42, p < 0.0001), whereas

there was no difference between normoxia and hyperoxia ($F = 3.22$, $p = 0.12$). For maximal work rates there was a significant difference between FIO_2 conditions ($F = 527.59$, $p < 0.0001$). Hypoxia induced a lower maximal WR than normoxia ($F = 398.18$, $p < 0.0001$) and hyperoxia ($F = 1207.52$, $p < 0.0001$). In addition, the maximal work rate during hyperoxia was higher than in normoxia ($F = 44.47$, $p = 0.0006$). The effect of FIO_2 upon RGET and maximal WR is presented in Fig. 5-5. It is clear that hypoxia had a much greater effect than did hyperoxia relative to the normoxic condition.

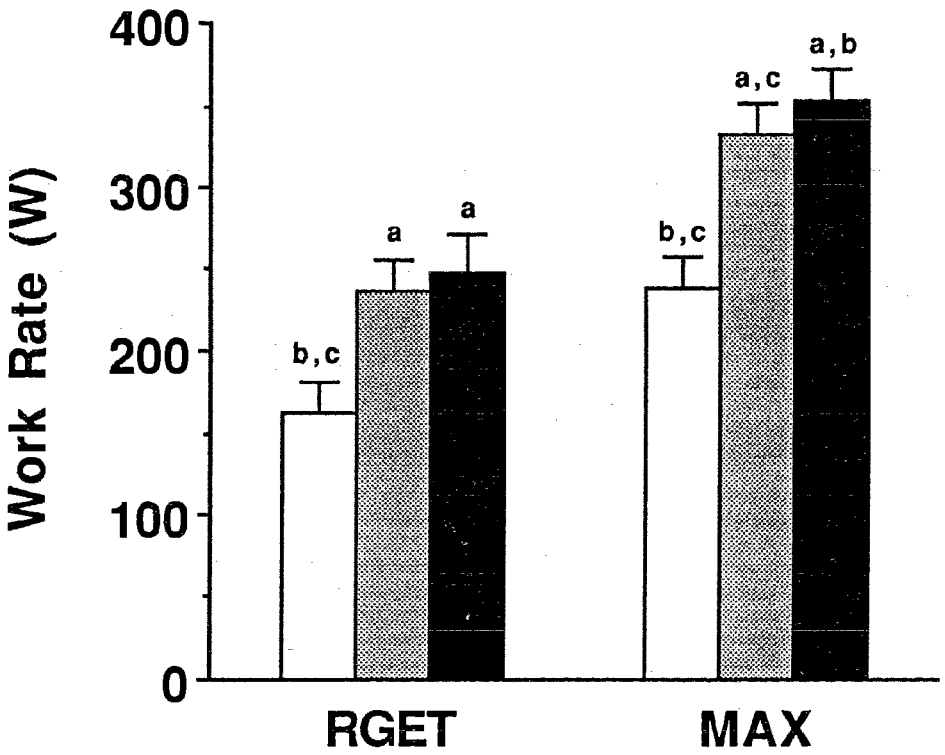

Fig. 5-5. Showing the work rate at which the respiratory gas exchange threshold (REGT) and exhaustion (MAX) occurred for each inspired oxygen condition. Empty bars are hypoxia, stipled bars are normoxia, and solid bars are hyperoxia. The error bars are ± S.D.
[a] significantly different from hypoxia, [b] significantly different from normoxia, and [c] significantly different from hyperoxia.

TABLE 5-4. *The group average RGET equivalent work rate expressed as a percentage of maximum work rate for each inspired oxygen condition.*

	Hypoxia	Normoxia	Hyperoxia
RGET (% max WR)	68.3	71.5	70.2
S.D.	7.5	5.0	5.3

When the RGET equivalent work rates were compared as a percentage of maximum work rate there was no significant difference between gases ($F = 1.80$, $p = 0.2076$) (Table 5-4).

The effect of time (*i.e.*, test 1 and test 2) or its interaction with either FIO_2 condition or slope was not significant for $\Delta\dot{V}O_2/\Delta WR$ relation nor was it significant for the RGET WR, maximum WR, and 30 W steady state and exhaustion values of $\dot{V}O_2$, $\dot{V}E$, and HR. This indicates a high degree of reproducibility of the results from one test the next.

TABLE 5-5. *The group average 30 W steady state values (± SD) for oxygen uptake, ventilatory rate, and heart rate during each inspired oxygen condition.*

	$\dot{V}O_2$ ($l \cdot min^{-1}$)	$\dot{V}E$ ($l \cdot min^{-1}$)	HR (beats $\cdot min^{-1}$)
Hypoxia	1.18 ± 0.11	41 ± 6[b,c]	120 ± 13[b,c]
Normoxia	1.17 ± 0.06	33 ± 4[a]	100 ± 7[a]
Hyperoxia	1.13 ± 0.06	33 ± 6[a]	99 ± 6[a]

[a] significantly different from hypoxia.
[b] significantly different from normoxia.
[c] significantly different from hyperoxia.

Steady state $\dot{V}O_2$ at 30 W was unaffected by FIO_2 (all F's < 2.34, all p's > 0.18). In contrast, $\dot{V}E$ and HR were higher during hypoxia than in either normoxia or hyperoxia (all F's > 29.04, all p's < 0.002). Comparisons of 30 W steady state $\dot{V}E$ and HR values between normoxia and hyperoxia did not attain significance (all F's < 0.99, all p's > 0.39). This was somewhat surprising, especially for HR, since pilot data at higher steady state work rates indicated that lower HR and $\dot{V}E$ occurred during hyperoxia. If the gas was switched from hyperoxia to normoxia

during exercise, HR usually showed a pronounced decrease within 2 breaths (Walsh and Banister, unpublished observations).

TABLE 5-6. *The group average maximum values (± SD) for oxygen uptake, ventilatory rate, and heart rate during each inspired oxygen condition.*

	$\dot{V}O_2$ ($l \cdot min^{-1}$)	$\dot{V}E$ ($l \cdot min^{-1}$)	HR (beats $\cdot min^{-1}$)
Hypoxia	$2.78 \pm 0.20^{b,c}$	$153 \pm 20^{b,c}$	$179 \pm 11^{b,c}$
Normoxia	$4.08 \pm 0.17^{a,c}$	169 ± 14^{a}	191 ± 8^{a}
Hyperoxia	$4.40 \pm 0.42^{a,b}$	167 ± 16^{a}	195 ± 7^{a}

[a] significantly different from hypoxia.
[b] significantly different from normoxia.
[c] significantly different from hyperoxia.

The effect of FIO_2 on $\dot{V}O_2$max was different from the FIO_2 effect on 30 W $\dot{V}O_2$. $\dot{V}O_2$max was lower with decreasing FIO_2 (all F's > 8.57, all p's < 0.03) (Table 5-6). For maximal $\dot{V}E$ and HR, the effect of FIO_2 was similar to that observed for the 30 W steady state data. Maximum $\dot{V}E$ and HR were lower in hypoxia compared with normoxia and hyperoxia (all F's > 9.52, all p's < 0.035). There were no differences in maximum $\dot{V}E$ and HR between normoxia and hyperoxia (all F's < 0.76, all p's > 0.45) (Table 5-6).

The IEMG data collected in the supplemental experiment are shown (Fig. 5-6 to 5-12). At higher work rates, IEMG data for each FIO_2 condition begin to separate (Fig. 5-6 and 5-7). The MedPF (Fig. 5-8) and MeanPF (Fig. 5-9) data were quite noisy and no trends between FIO_2 conditions were evident . Although less noisy, the zero crossing data also did not exhibit any trend between FIO_2 conditions (Fig. 5-10). The comparison of $\dot{V}O_2$/IEMG with WR is presented in Fig. 5-11 and 5-12. After 90 W (when $\dot{V}O_2$ is more linear), there is a clear separation of between FIO_2 conditions. Furthermore, there is a distinct decline of $\dot{V}O_2$/IEMG for each condition. This is because IEMG values increased about 6 fold from baseline whereas $\dot{V}O_2$ increased only about 4 fold.

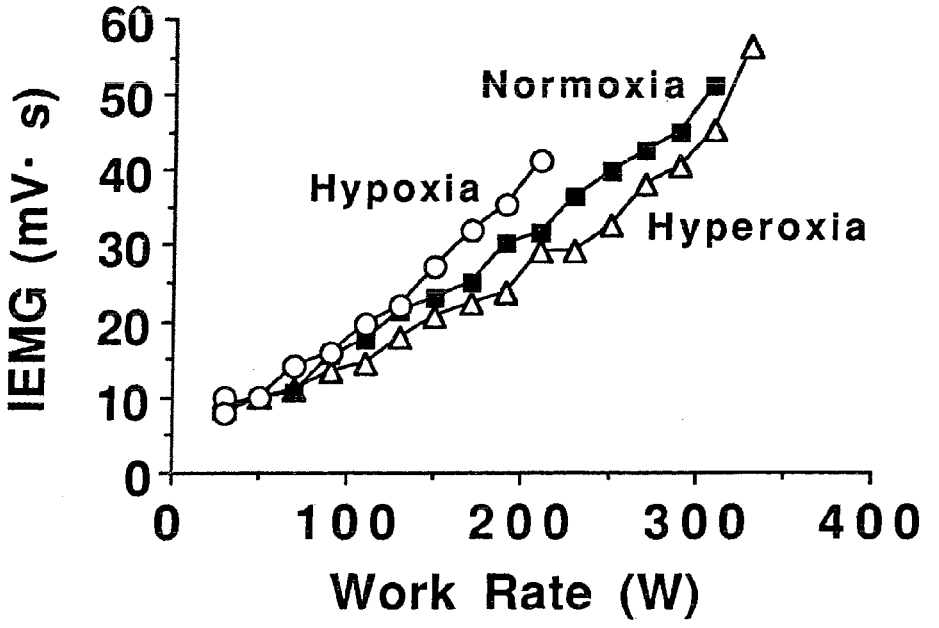

Fig. 5-6. Vastus medialis integrated EMG data collected during ramp work under 3 different FIO_2 conditions. The IEMG values are an average of 6 bursts.

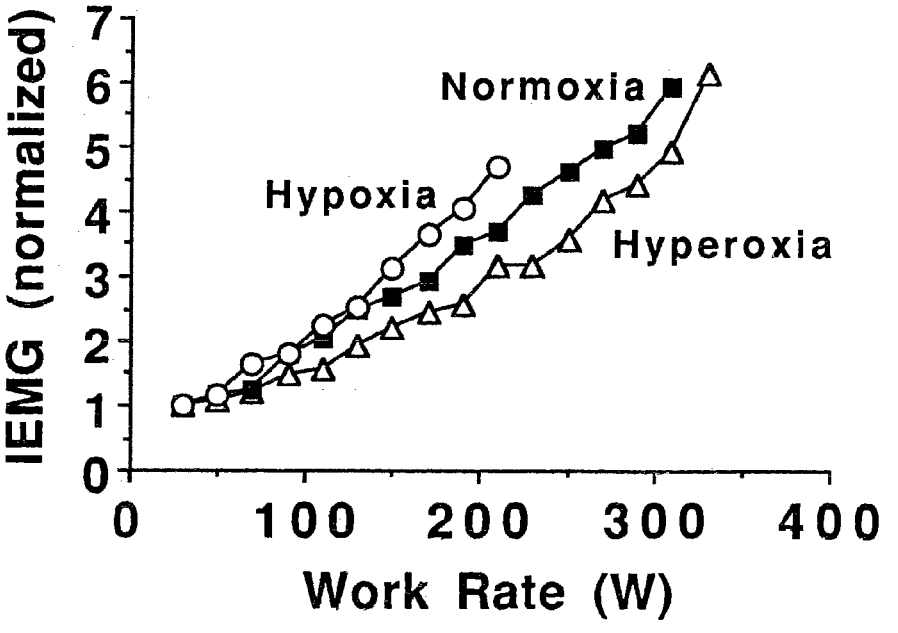

Fig. 5-7. Same as Fig. 5-6 except the integrated EMG data are normalized from 30 W values.

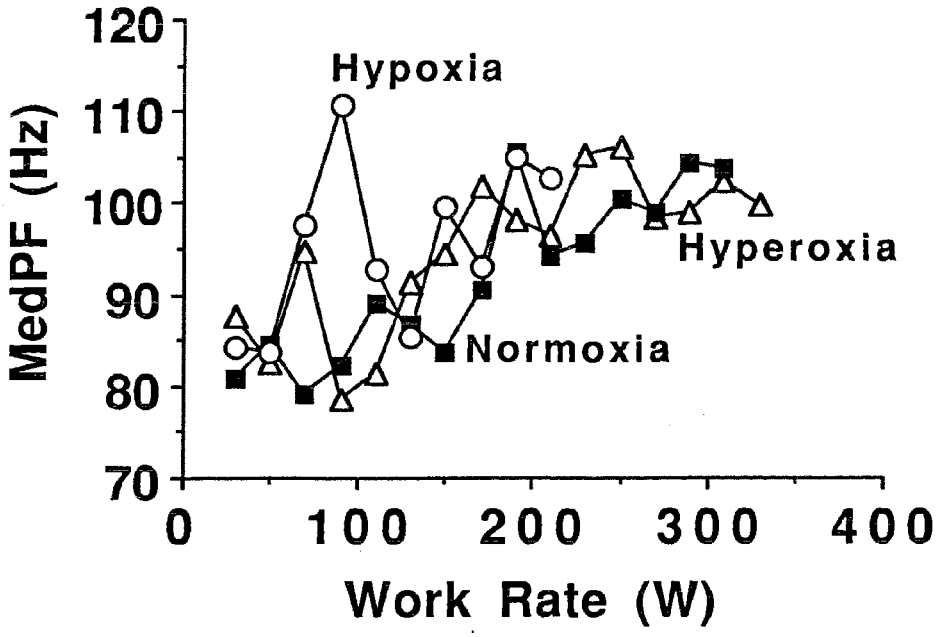

Fig. 5-8. The median power frequency (MedPF) of the vastus medialis EMG data collected during a work rate under 3 different inspired O_2 concentrations. The values are an average of 6 bursts.

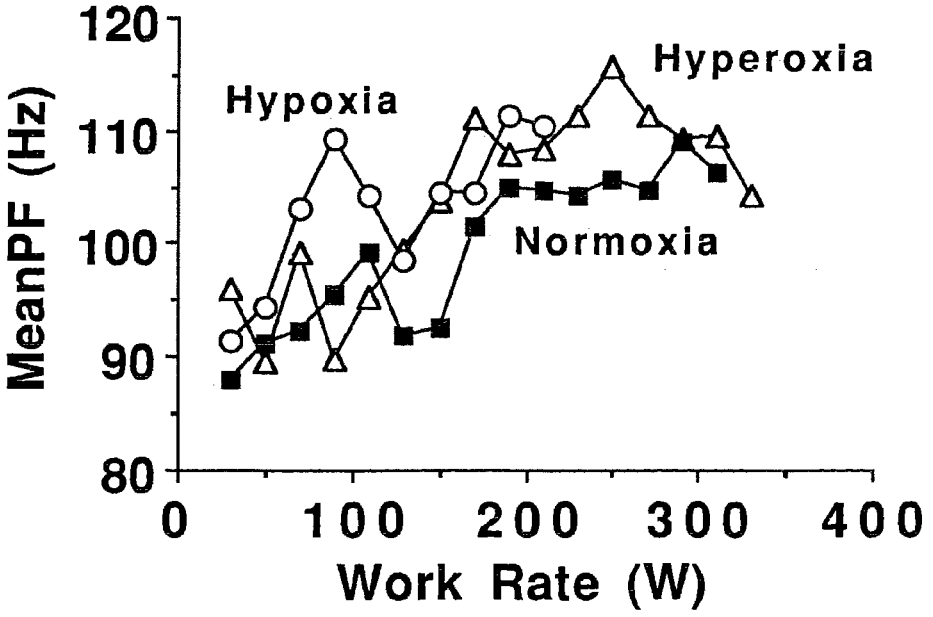

Fig. 5-9. The mean power frequency (MeanPF) of the vastus medialis EMG data collected during a work rate under 3 different inspired O_2 concentrations. The values are an average of 6 bursts.

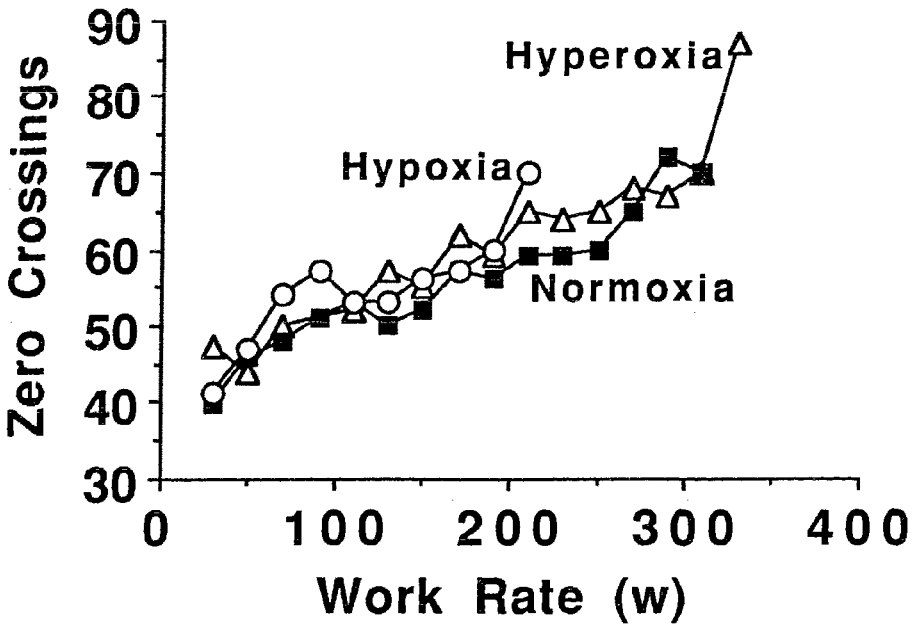

Fig. 5-10. The number of zero crossings observed at each work rate for each FIO_2 condition. The value are an average of 6 bursts.

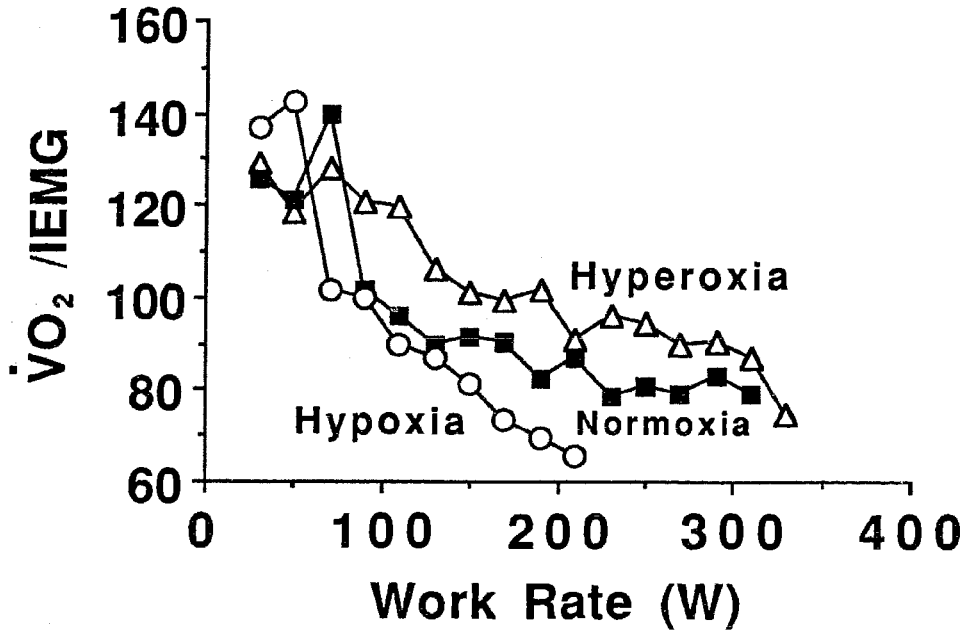

Fig. 5-11. The oxygen consumption per unit of EMG activity ($ml \cdot min^{-1} \cdot mV^{-1} \cdot s^{-1}$) at each work rate fo each FIO_2 condition. The IEMG values are an average of 6 bursts and the $\dot{V}O_2$ values are an average of breaths around each work rate selected.

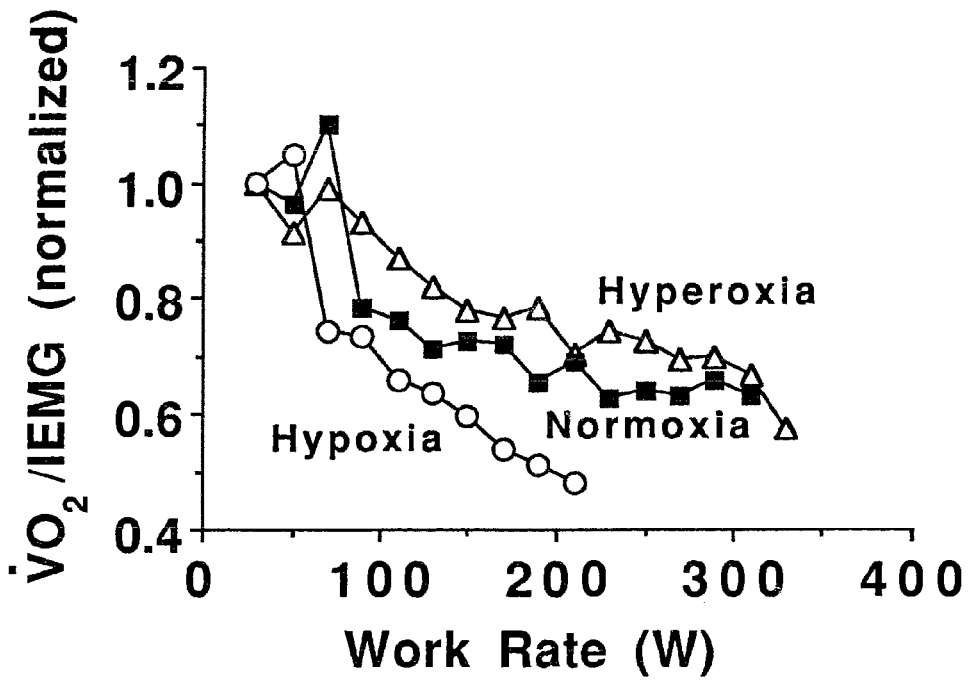

Fig. 5-12. Same as Fig. 5-11 except the data have been normalized from the 30 W values.

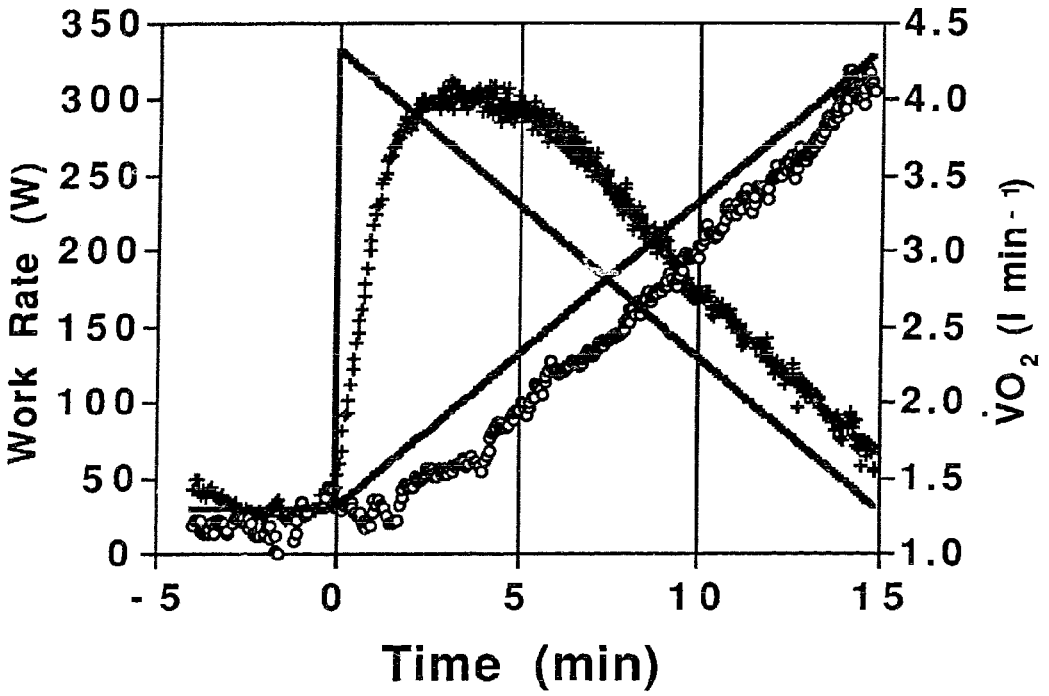

Fig. 5-13. $\dot{V}O_2$ and work rate versus time for both the positive ramp and step-negative ramp protocols. The data were smoothed with a 5 point moving average.

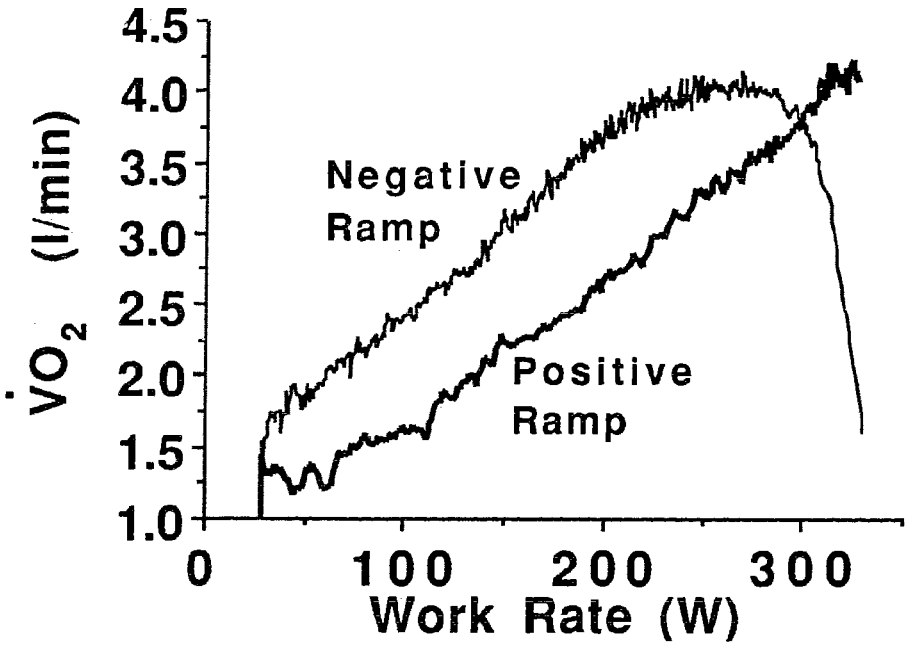

Fig. 5-14. The $\dot{V}O_2$ response to a positive ramp (thick line) and step-negative ramp (thin line) work rate protocol. The data were smoothed with a 5 point moving average.

There could be a concern that the change in $\dot{V}O_2$ slope which may be observed at the RGET is due to a time related effect. The step-negative ramp supplementary experiment demonstrated a reasonably linear $\dot{V}O_2$ response at work rates below about 187 W. There also appeared to be a change in slope at 138 W. The $\dot{V}O_2$ slope below 138 W was 10.4 ml $O_2 \cdot min^{-1} \cdot W^{-1}$. This value is very similar to the normoxic pre RGET $\dot{V}O_2$ slope of 10.3 ml $O_2 \cdot min^{-1} \cdot W^{-1}$. Thus time related effects do not appear to affect the $\dot{V}O_2$-WR relation for work rates below the RGET used in this study.

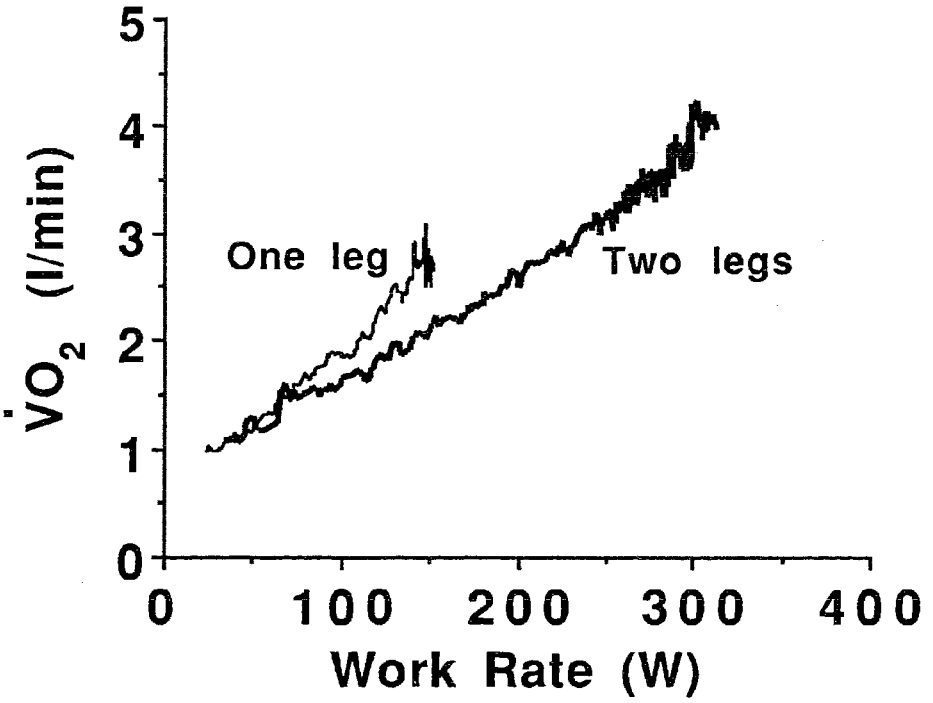

Fig. 5-15. The $\dot{V}O_2$ response to one-legged (from section 6, experiment 3) and two-legged ramp exercise (current experiment) to exhaustion in normoxia for one subject. The data were smoothed with a 5 point moving average.

The relation between oxygen uptake and work rate

The $\Delta\dot{V}O_2/\Delta WR$ relation was significantly affected by the FIO_2 condition. Below the respiratory gas exchange threshold (RGET), the hypoxic $\Delta\dot{V}O_2/\Delta WR$ slope was lower than that for either the normoxic or hyperoxic condition. The lower slope found in the present study during hypoxia indicates that the $\dot{V}O_2$ response to an unsteady state work rate condition is slower. This agrees with the results of Murphy *et al.* (1989) who compared normoxia with hypoxia during ramp exercise. Similar results have also been obtained for a moderate step increase in work rate (Linnarsson, 1974; Murphy *et al.*, 1989). On the contrary, Koike *et al.* (1990), who used carbon monoxide to reduce PaO_2, found a similar response to that of normoxia during ramp exercise. Wasserman and co-workers (Koike *et al.*, 1990; Wasserman and Koike, 1992) have used the similarity of the $\Delta\dot{V}O_2/\Delta WR$ relation below the RGET to indicate that O_2 is not limiting during this work rate range whereas they suggest that a reduction in $\Delta\dot{V}O_2/\Delta WR$ slope above RGET induced by carbon monoxide breathing indicates the onset of oxygen-limited metabolism. Having a slower $\dot{V}O_2$ response to unsteady state work does not imply an onset of oxygen-limited metabolism where none existed before. A lengthening of $\dot{V}O_2$ tau from 30 s to 40 s does not imply that the 40 s response is the only one that produces an anaerobic response to a ramp work rate protocol. Rather, unless the $\dot{V}O_2$ response is instantaneous to an increasing work rate, then there will always be a degree of oxygen-limited metabolism if a developed O_2 deficit is assumed to reflect such a condition.

Above the RGET, the $\Delta\dot{V}O_2/\Delta WR$ slope differed between all 3 FIO_2 conditions. A lower slope in hypoxia above the RGET compared with below the RGET indicates an even slower $\dot{V}O_2$ response in hypoxia. The pre and post RGET $\Delta\dot{V}O_2/\Delta WR$ were not different in normoxia. Hansen *et al.* (1988) suggest, that for a 30 $W \cdot min^{-1}$ ramp, the post RGET slope is not lower because the anaerobic contribution for maintaining the WR is much less than that for a 60 $W \cdot min^{-1}$ work rate

slope. As well, the post RGET slope does not increase as seen in a 15 $W \cdot min^{-1}$ ramp because the time is not long enough for such prominent phase 4 kinetics to be expressed. Thus the lack of change in $\Delta\dot{V}O_2/\Delta WR$ slope from pre to post RGET for a 30 $W \cdot min^{-1}$ ramp is explained by opposing oxygen-limited metabolism and phase 4 kinetic factors (Hansen *et al.*, 1988).

The $\Delta\dot{V}O_2/\Delta WR$ slope increase observed above RGET in hyperoxia appears contradictory. How can the slope increase if the muscle is supposed to be experiencing a greater degree of oxygen-limited metabolism? This could be a time related effect. Since exercise time was the longest for hyperoxia, one may expect some phase 4 $\dot{V}O_2$ kinetics to occur as suggested by Hansen *et al.* (1988) for slower ramp work rates. However, the ramp increment was the same as the other conditions and these data indicated that the increase was occurring well before the end of the exercise. Furthermore, when the ramp exercise was performed in reverse, the $\Delta\dot{V}O_2/\Delta WR$ slope near the end of the step-negative ramp is not different from the opposite positive ramp at work rates below RGET (Fig. 5-14). An increase in the slope of the $\Delta\dot{V}O_2/\Delta WR$ response has been observed in other circumstances. The average gain in the $\dot{V}O_2$ response to a work rate increase is about 10 ml $O_2 \cdot min^{-1} \cdot W^{-1}$ for two-legged cycling in normoxic conditions (Hansen *et al.*, 1987). A gain greater than this is not uncommon, but it is usually restricted to a smaller working muscle mass. In the one-legged training study (section 6.2) the $\Delta\dot{V}O_2/\Delta WR$ gain was much greater than this, especially above the RGET equivalent work rate (Fig. 5-15). Other studies using small muscle groups have also observed a gain greater than 10 $O_2 \cdot min^{-1} \cdot W^{-1}$ (*i.e.*, greater than two-legged cycling) (Andersen and Saltin, 1985; Casaburi *et al.*, 1992; Casaburi and Soll, 1992; Davies and Sargeant, 1974; Henriksson, 1977; Neary and Wenger, 1986; Sargeant and Davies, 1977; Saltin *et al.*, 1976; Stamford *et al.*, 1978; Toner *et al.*, 1983). The increased gain is most often apparent at a higher work rate and perhaps only above the lactate threshold work rate (Casaburi *et al.*, 1992). The higher gain with a smaller muscle mass has commonly been attributed to a greater use of stabilizing musculature (Sawka, 1986). However, for cyclic one-legged knee extensions with the subject secured to a seat, the need for muscular stabilization is small yet the O_2

cost per Watt range from 12 ml $O_2 \cdot min^{-1} \cdot W^{-1}$ at low work rates to 27 ml $O_2 \cdot min^{-1} \cdot W^{-1}$ at near maximal work rates (Andersen and Saltin, 1985). In addition, when both peak $\dot{V}O_2$ and O_2 deficit are determined for one and two-legged cycling, the peak one-legged $\dot{V}O_2$ is 80 % of the corresponding two-legged value whereas the one-legged O_2 deficit is only about 50 % of the two-legged value (Weyand *et al.*, 1993). This indicates that the additional higher $\Delta\dot{V}O_2/\Delta WR$ observed during one-legged exercise is not due only to muscle recruitment for stablisation purposes. From this it may be concluded that much of the increase $\Delta\dot{V}O_2/\Delta WR$ gain observed for a smaller working muscle mass is generated by the working muscle itself. Thus the one-legged response in normoxia may be similar to two legs in hyperoxia. This observation is supported by the fact that the one-legged $\Delta\dot{V}O_2/\Delta WR$ response is unchanged in hyperoxia (Davies and Sargeant, 1974).

Koike *et al.* (1990) demonstrated a reduction in the $\Delta\dot{V}O_2/\Delta WR$ above the RGET induced by carbon monoxide breathing; this effect is analogous to the hypoxic condition of the present study. The authors used this evidence to suggest that the RGET is truly an anaerobic threshold. The hypothesized increase in anaerobic metabolism is suggested to be the result of a perfusion limitation (Wasserman *et al.*, 1990; Wasserman and McIlroy, 1964). This implies that an increase in extraction would not be capable of offsetting a relative decrease in delivery. Thus for some capillaries either all the O_2 is extracted before the end of the capillary or the transit time is too short to deliver the O_2 required by the muscle. A perfusion limitation hypothesis is supported by a variety of studies. For one-legged knee extension exercise there is not a perfusion limitation because the muscle mass involved is very small and thus leg blood flow continuously increases during rhythmical contractions to exhaustion as work rate is increased (Andersen and Saltin, 1985; Rowell *et al.*, 1986). Adding arm exercise to ongoing leg exercise reduces leg blood flow (Secher *et al.*, 1977). This effect manifests itself well below $\dot{V}O_2max$, supporting a perfusion limitation rationale for 2-legged cycling at submaximal work rates despite a metabolic vasodilatory effect that should increase with increasing work rate. A greater flow restriction at a higher work rate may partly explain the lower $\Delta\dot{V}O_2/\Delta WR$ observed for a 60 $W \cdot min^{-1}$ than a 15 $W \cdot min^{-1}$ ramp

protocol (Hansen *et al.*, 1988). If a significant increase in vasoconstriction occurs at the RGET then total peripheral resistance is increased. If blood pressure does not increase to counter the increased resistance, then the rate of $\dot{Q}$ increase should be diminished. During ramp exercise the rate of increase of blood pressure is decreased at or above the RGET (Walsh, unpublished observation) suggesting a reduction in the perfusion rate. The nonlinear increase in IEMG observed in the supplemental study is analogous to the increase in EMG activity when blood flow is restricted while the muscle continues performing at the same power output (Moritani *et al.*, 1992). If muscle perfusion is reduced by lower body positive pressure, blood lactate concentration is increased (Eiken and Bjurstedt, 1987; Sundberg and Kaijser, 1992). Similarly, if muscle perfusion is enhanced with lower body negative pressure, blood lactate concentration is reduced (Eiken and Bjurstedt, 1985). It is assumed that metabolites, important for the regulation of energy metabolism, may change comparably with blood lactate.

The training induced increase in the RGET could be accounted for in a perfusion hypothesis by an increased $\dot{Q}$ and capilliarity (increasing mean transit time) increasing the WR until a perfusion limitation occurred. A reduction in perfusion does not necessarily imply some muscle fibres are anaerobic, but rather that since $\dot{V}O_2$ is still increasing with increasing work rate, more demand is put on extraction than delivery in attaining the required $\dot{V}O_2$. When this occurs, a greater rate of change in intracellular metabolites takes place in order to reduce mitochondrial PO_2 and thus maintain an adequate oxygen gradient into the muscle cell. A training induced increase in $\dot{Q}$ and capillarity, and thus muscle perfusion, may delay the occurance of a metabolic threshold.

Other evidence argues against a perfusion limitation inducing an anaerobic threshold and a reduction in $\Delta\dot{V}O_2/\Delta WR$. In the present experiment, when hyperoxic air was inspired during ramp exercise an RGET was still observed yet $\Delta\dot{V}O_2/\Delta WR$ increased. An increase in positive pressure upon working muscles (lower body positive pressure) does not alter the steady state $\dot{V}O_2$ because the reduced perfusion is offset by a greater extraction (Sundberg and Kaijser, 1992). For one-legged knee extension there is not a perfusion limitation yet a nonlinear increase in both blood

lactate and catecholamines still occurs (Andersen and Saltin, 1985; Rowell *et al.*, 1986). Rather than exclude the perfusion hypothesis as a mechanism inducing the RGET and changing the $\Delta\dot{V}O_2/\Delta WR$ relation, it is more prudent to suggest that another mechanism or mechanisms also have a mitigating influence.

A reduction in efficiency may explain some of the effects noted above. As with the perfusion hypothesis, an efficiency hypothesis must be related to muscle unit recruitment because only small differences in blood lactate and $\Delta\dot{V}O_2/\Delta WR$ are observed below the RGET after which these differences become prominent and significant (Kioke *et al.*, 1990). A reduction in efficiency implies that a greater oxygen cost is required for a given amount of work done. For 2-legged exercise, the increased oxygen cost is commonly restricted to work rates above the RGET for steady state exercise (Casaburi *et al.*, 1989a), for a slow ramp (15 $W \cdot min^{-1}$) while breathing normoxic air (Hansen *et al.*, 1988), and for ramp exercise above the RGET while breathing hyperoxic air (present study).

Efficiency in ergometric exercise is affected by two mechanisms: the coupling of oxygen to high energy phosphates and the coupling of high energy phosphate hydrolysis to work generating mechanisms and to ion pumping. Any reduction in efficiency observed in the above processes may be due, in part, to the accumulation of fatigue (as indicated by an early force reduction in a maximal voluntary contraction during submaximal contractions) that occurs very early during exercise (Vollestad *et al.*, 1988). The metabolic consequences of fatigue may cause a reduction in the free energy of ATP (Dawson *et al.*, 1980; Kammermeier, 1987) and would lead to a reduction in the coupling of ATP to force generation or ion pumping or both if the reduction in free energy is great enough. This effect was much more prominent in muscle units recruited above the RGET in the present study because the $\Delta\dot{V}O_2/\Delta WR$ relation did not change for low work rates when a step-negative ramp was performed (Fig. 5-13 and 5-14). This implies that a certain amount of efficiency reduction observed above RGET is intrinsic to the muscle unit independent of accumulating fatigue.

A threshold decrease in efficiency can account for the increased $\Delta\dot{V}O_2/\Delta WR$ observed during ramp exercise breathing hyperoxia and the phase 4 kinetics observed at any work rate greater than the RGET equivalent work rate during step exercise.

If the high $\Delta\dot{V}O_2/\Delta WR$ values observed for small working muscle masses represents the true O_2 cost of doing work, then it is perhaps a perfusion limitation of a larger working muscle mass that opposes the increasing $\Delta\dot{V}O_2/\Delta WR$ expected for 2-legged exercise based on the extrapolation of a small muscle mass effective $\Delta\dot{V}O_2/\Delta WR$ gain.

The $\Delta\dot{V}O_2/\Delta WR$ increase above the RGET in hyperoxia could be due either to a faster response of the system or an increase in $\Delta\dot{V}O_2(ss)$ demanded or both. The response might be faster because less change in metabolite concentration is required to generate the same $\dot{V}O_2$. An increase in $\Delta\dot{V}O_2(ss)$ demanded may occur because of a decrease in efficiency which may be related to motor unit recruitment. No matter what the underlying mechanism of a $\Delta\dot{V}O_2/\Delta WR$ increase in hyperoxia, the increase suggests that the working muscles are capable of utilising more O_2 if it is provided. This implies a greater degree of O_2 limitation above the RGET for normoxic and hypoxic 2-legged ramp exercise.

Maximum ramp exercise cardiorespiratory values

The maximal work rate increased as FIO_2 increased from 0.12 to 0.40 in the present experiment. This finding is predictable from previous studies (Murphy *et al.*, 1989; Yoshida *et al.*, 1987). More interestingly, the work rate equivalent to the RGET changed in a similar fashion. Thus the RGET, relative to maximal work rate, was the same for all 3 FIO_2 conditions. This has been demonstrated previously in the comparison between hypoxia and normoxia (Murphy *et al.*, 1989; Yoshida *et al.*, 1987), however, the significance of this effect has been underemphasized. That the RGET occurs at the same percentage of $\dot{V}O_2max$ under a variety of conditions demonstrates that a physiological response may be dependent on both a relative and absolute change in metabolism.

In normoxia, a subject may have a $\dot{V}O_2$max of 4 l·min^{-1} and this may take a 30 mM increase in [C] to generate the oxygen flux (Fig. 5-16). If the subject exercised at 2 l·min^{-1} $\dot{V}O_2$ in normoxia it would only take a 15 mM change in [C] to maintain the oxygen flux whereas in hypoxia, it would take a 30 mM change in [C] to generate the same 2 l·min^{-1}. On a relative basis,

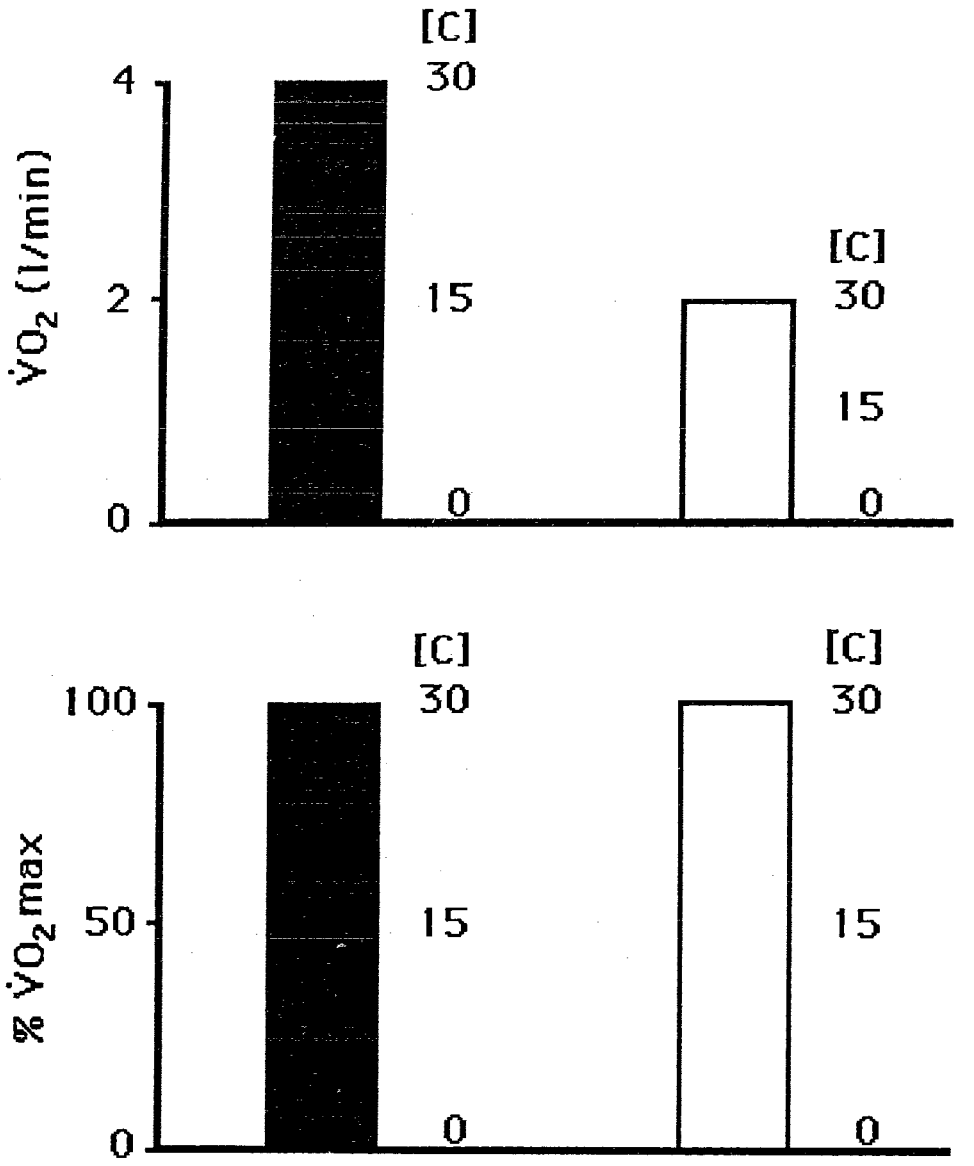

Fig. 5-16. A schematic representation of $\dot{V}O_2$ (top) and % $\dot{V}O_2$max (bottom) to creatine concentration ([C]: mM) during exercise in normoxia (filled bar) and hypoxia (empty bar).

in order to induce a maximal O_2 flux into a cell requires a maximal change (30 mM) in creatine or some other potential oxidative regulator. For the example in Fig. 5-16, the lactate threshold is assumed to occur at 50 % $\dot{V}O_2$max. This suggests the lactate threshold may be influenced by an absolute change in a metabolic regulator (*e.g.*, Δ15 mM [C]) (see section 6.1.3.2 for further discussion). This may rule out substrate supply as a possible mechanism inducing a lactate threshold. It has been postulated that the lactate threshold may occur when flux of fats into the TCA cycle reaches a maximum and thereafter glycolysis becomes more prominent as a substrate supplier for oxidation (see Walsh and Banister (1988) for a review). Maximal flux through the fatty acid oxidation cycle should not be directly influenced by PO_2 yet the lactate threshold occurs at a much lower work rate in hypoxia. The rate of fatty acid oxidation should be very different at the work rate at which the lactate threshold occurs in normoxia and hypoxia. This excludes a maximal flux of fat supply as an inducer of the lactate threshold.

The maximum values for $\dot{V}O_2$, $\dot{V}E$, and HR were less in hypoxia than in the other FIO_2 conditions in the present experiment. This was reflec d in a shorter ramp exercise time. Since $\dot{V}E$ and HR are potentially capable of greater contributions, this suggests $\dot{V}O_2$max was limited to a greater degree by peripheral mechanisms rather than by either a central pulmonary or circulatory functioning or both. Maximum $\dot{V}E$ and HR were not different between normoxia and hyperoxia. The higher $\dot{V}O_2$max in hyperoxia would be due to a greater a-vO_2 difference because of the higher PaO_2.

Baseline cardiorespiratory values

$\dot{V}O_2$ at 30 W was not different between the 3 FIO_2 conditions. This result is similar to other studies (Katz and Sahlin, 1987; Linnarsson *et al.*, 1974; Murphy *et al.*, 1989; Nakazono and Miyamoto, 1987; Welch and Pedersen, 1981; Yano and Asano, 1984). Since arterial O_2 content would be different in the 3 FIO_2 conditions, compensation in another part of the O_2 delivery and utilisation pathway must have occurred especially if the $\dot{T}O_2/\dot{V}O_2$ (the amount of O_2 transported compared with the amount of O_2 utilized) was reduced to below about 2.5 (Hirschl *et al.*, 1992).

Such a compensation could be accomplished by an increase in blood flow during reduced FIO_2 as indicated by a higher HR in the present study and greater cardiac output in other studies similarly conducted (Murphy *et al.*, 1989; Nakazono and Miyamoto, 1987).

Supplemental EMG study

EMG amplitude of the vastus lateralis is linear with WR for individual 1 minute steady state conditions from 20 to 100 % $\dot{V}O_2max$ (Petrofsky, 1979). In the present study, using a ramp work rate protocol, IEMG demonstrates a nonlinear increase as WR increases. The WR or $\dot{V}O_2$ at which IEMG increases at a faster rate has been validated as a noninvasive measure of the anaerobic threshold (Matsumoto *et al.*, 1991; Nagata *et al.*, 1981; Viitasalo *et al.*, 1985). At a higher WR, the additional IEMG signal indicates that a greater muscle activation is required to generate a given power output. This indicates that, during ramp exercise, the muscle is beginning to fatigue. This is even more evident when comparing the IEMG for different FIO_2 conditions at the same work rate. The greater IEMG recorded with increasing severity of the FIO_2 condition is very striking. The greater IEMG response observed as FIO_2 decreased, especially at a work rate above RGET, indicates an even greater activation of the muscle is required to generate the same power output. Detailed EMG analysis has not been applied to the study of rhythmic contractions as it has been for static contractions. For a repeated submaximal isometric contraction (6 s on and 4 s off), a force decay in both maximal voluntary contraction and electrically stimulated maximal contraction is very evident by 1 min (Vollestad *et al.*, 1988). These data support the probability that the nonlinear increasing IEMG observed in the present study is due to accumulating fatigue within the muscle. When blood supply to a muscle performing a constant power output is interrupted, EMG activity increases (Moritani *et al.*, 1992) suggesting a link between O_2 delivery and the onset of a nonlinear increase in EMG activity. Whether the greater muscle activation is due to recruitment of more motor units resulting in less power output per motor unit or due to greater activation of the same number of motor units under each FIO_2 condition for a given work rate cannot be discerned from the present experimental data. However, when repeated submaximal isometric contractions

are examined, recruitment of additional motor units contributes substantially more than does rate coding to the increase in EMG (Bigland-Ritchie *et al.*, 1986a).

As FIO_2 increased from 0.12 to 0.40, the maximal IEMG increased in the present experiment. This suggests that the common high altitude or hypoxic method of endurance training would not be as beneficial as training in normoxia and even more so in hyperoxia. At any percentage of $\dot{V}O_2max$, the IEMG is higher in the higher FIO_2 condition. This indicates that more muscle fibres are being trained in the higher FIO_2 condition because the work rate is greater. It is logical to assume that the more muscle fibres being trained for the same relative intensity, the better the training effect. Thus from a muscle perspective, the greater the FIO_2 condition, the greater the training effect.

There were no observable differences for the EMG indices of MedPF, MeanPF, and ZC for the 3 FIO_2 conditions. Of these 3 measures, MedPF is the best indicator of motor unit firing frequency, and ZC is the best indicator for motor unit action potential velocity decrease (Hagg, 1991). If these indicators are sensitive and valid, the motor unit recruitment pattern was not altered between FIO_2 conditions as would be evident by motor unit firing changes. Furthermore, the spread of the action potential across the muscle was not affected by changing FIO_2. Similar nonsignificant changes in MeanPF have been observed with ischaemia even though EMG amplitude was affected (Gerdle and Fugl-Meyer, 1992).

The $\dot{V}O_2$/IEMG comparison with work rate is very intriguing. There is a clear difference in this ratio between FIO_2 conditions. This is mostly due to the difference in IEMG below the REGT and a difference in both $\dot{V}O_2$ and IEMG above the RGET. The decline in $\dot{V}O_2$/IEMG at work rates well below RGET is very surprising. Examination of $\dot{V}O_2$/IEMG occurs very infrequently in the literature. An examination of data from Bigland-Ritchie and Woods (1974) indicates the $\dot{V}O_2$/IEMG does not change for steady state exercise. Although not indicated, the work rates used by Bigland-Ritchie and Woods (1974) were most likely below the RGET. The larger work rate range and the different work rate stimulus presented by the ramp protocol in the present study

probably account for the difference between their results and the present study. The $\dot{V}O_2$/IEMG decline in the present study suggests that oxygen utilisation is not keeping pace with muscle activation even at a low work rate. This phenomenon deserves further investigation.

6.0 REGULATION OF THE NONSTEADY STATE OF THE $\dot{V}O_2$ RESPONSE TO A CHANGE IN WORK RATE

6.1 REVIEW OF LITERATURE

6.1.1 INTRODUCTION

Kinetic analysis of the $\dot{V}O_2$ response to a step increase in work rate involves calculating the $\dot{V}O_2$ each breath and determining the rate and magnitude of the $\dot{V}O_2$ change from a baseline to the steady state induced by the work rate perturbation. The phase 2 $\dot{V}O_2$ response resembles a saturating exponential rise from baseline to steady state (Fig. 2-3). There is a series of processes which link the input (*i.e.*, an increase in ATP hydrolysis) to the output (*i.e.*, the $\dot{V}O_2$ response measured at the mouth). These processes include:

1) An increased WR causes increased ATP hydrolysis (**INPUT**).

2) The mitochondrion is stimulated to produce ATP thus utilising O_2.

3) A decrease in PO_2 at the mitochondrion is created.

4) The pressure gradient between the mitochondrion and the blood increases.

5) Neural and humoral mechanisms increase blood flow to the working muscle.

6) More O_2 flows into the active muscle cell.

7) The subsequent decrease in venous PO_2 is transferred to the lungs.

8) A greater rate of O_2 enters the blood from the air.

9) Air is exhaled with less O_2 and this decrement is measured (**OUTPUT**).

Each step of this sequence may be divided into 2 main processes: convection or diffusion. This is dependent on whether they involve primarily a delivery component (*i.e.*, convection) or a pressure differential component (*i.e.*, diffusion). Step 1, the input, is the forcing function of the system. Steps 2, 3, 4, 6, and 8 involve primarily a diffusion process, whereas steps 5, 7, and 9

involve convection. These steps all contribute to the $\dot{V}O_2$ response. It is amazing that $\dot{V}O_2$ appears to be a linear function of WR and resemble first order exponential kinetics despite the complexity of the intervening steps.

One possible reason for the apparent simplistic first order $\dot{V}O_2$ response, despite the on-going complex serial and parallel processing, is that perhaps only one step is rate limiting. This concept is supported by numerous authors, but controversy has arisen as to which step is limiting. Some investigators suggest that O_2 delivery to the working muscle is the rate-limiting step (see Hughson, 1990) whereas others suggest that the ability of the active muscle to utilise O_2 is rate limiting (Whipp and Mahler, 1980). Thus, like the debate about the locus limiting $\dot{V}O_2max$, a similar debate applies to which locus regulates $\dot{V}O_2$ kinetics: are $\dot{V}O_2$ kinetics limited centrally (*i.e.*, by convective mechanisms) or peripherally (*i.e.*, by diffusive mechanisms)?

The $\dot{V}O_2$ response to a work rate forcing function such as an impulse, step, or ramp has most often been represented by an exponential equation. The family of exponential curves has the unique property that the rate of change of a response is proportional to the error signal. Thus the rate of increase of the $\dot{V}O_2$ response is proportional to the difference between the current $\dot{V}O_2$ level and the steady-state level ultimately demanded by the forcing function. If a single step in the overall $\dot{V}O_2$ response process is rate limiting then a first order exponential $\dot{V}O_2$ response may be anticipated. If 2 or more first-order steps of the $\dot{V}O_2$ response process are limiting then the $\dot{V}O_2$ response would be expected to exhibit a sigmoid form (Jones, 1973). Rarely, however has such a $\dot{V}O_2$ response been documented. Whipp and Mahler (1980) report a sigmoidal response to a step increase in work rate from unloaded pedalling to a work rate above the lactate threshold. Cerretelli *et al.* (1977) also recorded such a response for arm cranking equivalent to an O_2 cost of about 1.0 $l \cdot min^{-1}$. One reason for the rarity of this sigmoidal-shaped $\dot{V}O_2$ response may be that it maybe masked by the phase 1 response.

6.1.2 EVIDENCE CONCERNING CENTRAL LIMITATIONS OF $\dot{V}O_2$ KINETICS

The importance of a central mechanism regulating $\dot{V}O_2$ kinetics is that it is possible to to meet an increase in $\dot{V}O_2$ demand entirely by increasing the delivery of O_2 and hence there is no change in the a-vO_2 difference. This is evident from the Fick equation:

$$\dot{V}O_2 = \dot{Q} \cdot (CaO_2 - CvO_2) \quad \text{.....(6-1)}$$

where $\dot{Q}$ is the cardiac output and CaO_2 and CvO_2 are the arterial and venous concentrations of oxygen, respectively.

Three methods are commonly used to identify the site(s) limiting the $\dot{V}O_2$ response to a work rate stimulus. One approach is to identify the physiological compartment with the same response characteristics as the $\dot{V}O_2$ response. Another approach is to modify a process involved in the body's regulation of O_2 utilisation and observe the effect produced on the $\dot{V}O_2$ response. These two methods involve acute experimental observation. A third approach is by a chronic intervention. For instance, it is known that an endurance trained person has a faster $\dot{V}O_2$ response than a sedentary person (Hagberg *et al.*, 1978; Sietsema *et al.*, 1989; Whipp and Wasserman, 1972) and that training decreases $\dot{V}O_2$ tau whether it is compared at the same absolute (Bang, 1936; Cerretelli *et al.*, 1979; Hickson *et al.*, 1978; Yoshida *et al.*, 1992) or relative WR (Hickson *et al.*, 1978). From such chronic studies, a change in $\dot{V}O_2$ tau, may be correlated with a change in another variable measured concurrently.

6.1.2.1 CARDIAC OUTPUT

When the time course of $\dot{Q}$ and $\dot{V}O_2$ are measured concurrently in response to a work rate change, $\dot{Q}$ may be inferred to regulate the $\dot{V}O_2$ response if $\dot{Q}$ tau is approximately equal to $\dot{V}O_2$ tau. Several studies that have measured the time course of cardiac output during an acute work rate transition question the limiting effect on the $\dot{V}O_2$ response suggested for $\dot{Q}$ in response to a work stimulus. Cardiac output displays a faster kinetic response than $\dot{V}O_2$ at the onset of exercise

(Ceretelli *et al*, 1966; Davies *et al.*, 1972; De Cort *et al.*, 1991; Eriksen *et al.*, 1990; Grucza *et al.*, 1990; Shindell *et al.*, 1977). Although Inman *et al.* (1987) found the $\dot{V}O_2$ time constant for a step increase in work rate to be longer than that of cardiac output (25 vs 17 s) these authors used a model to predict the $\dot{V}O_2$ kinetics at the tissue level and estimated the time constant of this process to be 15 s. Based on the similarity of the tissue $\dot{V}O_2$ time constant with $\dot{Q}$ tau, the authors concluded that the delivery process limits oxygen kinetics.

Recent studies have taken advantage of improved technology and recorded $\dot{Q}$ on a beat-to-beat basis. This allows a more accurate determination of $\dot{Q}$ tau than previous methods constrained to using averaging techniques. Using impedance cardiography, Grucza *et al.* (1990) determined a $\dot{Q}$ tau of 17 s for a step change to 50 % $\dot{V}O_2$max during cycle ergometry. Doppler ultrasound has been used to determine the unsteady state $\dot{Q}$ response to a small step increase in work rate during supine cycle ergometry (Eriksen *et al.*, 1990). A very fast $\dot{Q}$ tau of about 10 s was calculated for this protocol. Thus the beat-to-beat measure of $\dot{Q}$ indicates a 2-4 fold faster $\dot{Q}$ response than is usually observed for $\dot{V}O_2$. Yoshida *et al.* (1993) simultaneously measured $\dot{V}O_2$ (breath-by-breath) and $\dot{Q}$ (impedence cardiography) for WR transitions from rest to 50, 75, and 100 W and from 25 W to 50, 75, and 100 W. They found the phase 2 $\dot{V}O_2$ tau and $\dot{Q}$ tau to be the same for all exercise transitions. This would indicate that delivery factors strongly influence $\dot{V}O_2$ kinetics. However, the authors concluded that $\dot{V}O_2$ kinetics were regulated peripherally despite their evidence because $\dot{V}O_2$ tau was the same for both rest to 50, 75, and 100 W and from 25 W to 50, 75, and 100 W.

Studies which modify $\dot{Q}$ have demonstrated an alteration in $\dot{V}O_2$ tau. Reducing steady-state $\dot{Q}$ by 2.0 $l \cdot min^{-1}$ with beta-adrenergic blockade lengthens $\dot{V}O_2$ tau and HR tau at the onset of exercise (Petersen *et al.*, 1983). $\dot{V}O_2$max and the ventilatory threshold were simultaneously reduced by 5 and 23 % respectively. The increased $\dot{V}O_2$ tau demonstrates the strong influence that $\dot{Q}$ has upon $\dot{V}O_2$ kinetics. Unfortunately, in the above experiment, an absolute WR was used in determining $\dot{V}O_2$ tau. For most subjects, the step increase in WR was below the lactate threshold

without beta-blockade and equal to or above the subject's lactate threshold with beta-blockade. This will add a significant phase 4 component to the $\dot{V}O_2$ response during work when exercising under beta-blockade conditions. Petersen *et al.* (1983) used a single exponential model to determine $\dot{V}O_2$ tau. Such a procedure will result in a longer estimate of $\dot{V}O_2$ tau when the phase 4 component of the $\dot{V}O_2$ response is present. Hughson (1984) also determined a longer $\dot{V}O_2$-ON response with beta-adrenergic blockade for a 0-100 W step increase in work rate but did not find any alteration in the rate of the heart rate response. As Hughson (1984) suggests, until the kinetics of cardiac output and muscle blood flow can be measured concurrently with $\dot{V}O_2$ kinetics, one can only speculate that O_2 transport regulates the $\dot{V}O_2$ response.

Cardiac output kinetics are thought to be slower in the supine than upright position. Hughson *et al.* (1991) determined that $\dot{V}O_2$ kinetics are also slower in the supine position. However, $\dot{V}O_2$ kinetic lengthening was due to a change in the phase I response and lag time. Exercise position did not change the time course of the phase 2 response.

$\dot{V}O_2$ kinetics are slowed by bed rest (Convertino *et al.*, 1984). After 7 days of bed rest this effect is only observed during upright and not supine exercise. It may be surmised from this that $\dot{V}O_2$ kinetics are slowed by a convection mechanism (*i.e.*, cardiac output) and not by a diffusion mechanism (*i.e.*, a change in a-vO_2 difference). Ventilatory kinetics were not affected.

In a subject with a cardiac pacemaker, it is possible to control the heart rate response (but not cardiac output) to a step increase in work rate. At the onset of exercise, heart rate was kept constant at 50 beats·min^{-1} or increased to 100 beats·min^{-1}. The phase 2 $\dot{V}O_2$ time constant was actually slower when the the heart rate was increased (Casaburi *et al.*, 1989c). Similarly, when blood flow to dog gastrocnemius muscle was experimentally increased immediately to the predetermined steady state at exercise onset rather than allowing it to follow a normal time course, the phase 2 $\dot{V}O_2$ response was the same or slower (Corsi *et al.*, 1975). These direct interventions indicate that cardiac output plays only a minor role in regulating $\dot{V}O_2$ kinetics. Modeling the

pulmonary $\dot{V}O_2$ response also indicates that altering $\dot{Q}$ tau has little influence on $\dot{V}O_2$ kinetics (Barstow *et al.*, 1990).

If $\dot{Q}$ regulates $\dot{V}O_2$ kinetics and training reduces $\dot{V}O_2$ tau it is logical to assume that $\dot{Q}$ tau also shortens with training. This has not been specifically investigated, however, Yoshida *et al.* (1992) have demonstrated a training induced decrease in the time constant for both heart rate and $\dot{V}O_2$.

6.1.2.2 OXYGEN DELIVERY TO THE WORKING MUSCLE

Cardiac output is one of several physiological processes contributing to O_2 delivery to working muscle. The amount of O_2 transported by arterial blood to the systemic circulation is also dependent on the hemoglobin concentration ([Hb]: $gm \cdot ml^{-1}$) and the degree to which the hemoglobin is saturated with O_2 (SaO_2: fractional percent).

$$\dot{T}O_2 = \dot{Q} \cdot [Hb] \cdot SaO_2 \cdot 1.37 \quad (6\text{-}2)$$

The constant 1.37 specifies the amount of O_2 that binds to hemoglobin ($ml \cdot gm^{-1}$). The transport of O_2 ($\dot{T}O_2$: $ml \cdot min^{-1}$) to muscle rather than $\dot{Q}$ ($l \cdot min^{-1}$) has been suggested as a regulated variable (Ferretti *et al.*, 1992; Saltin *et al.*, 1986). Thus an alteration in one parameter determining $\dot{T}O_2$ may be compensated for by an offsetting change in another parameter of Eqn. 6-2.

If blood oxygen is increased by breathing hyperoxic air there is a corresponding reduction in $\dot{Q}$ to maintain $\dot{T}O_2$ constant (Hughes *et al.*, 1968; Saltin *et al.*, 1986). Similarly, if the O_2 content of the blood is reduced by breathing hypoxic air, etc., there is a compensating increase in $\dot{Q}$ to maintain $\dot{T}O_2$ constant (Bender *et al.*, 1988; Hughes *et al.*, 1968; Rowell *et al.*, 1986; Saltin *et al.*, 1986; Stenberg *et al.*, 1966). Furthermore, at a submaximal work rate, $\dot{T}O_2$ remains constant despite a large variation in [Hb] (11.8-17.2 $gm \cdot dl^{-1}$) (Ferretti, *et al.*, 1992; Saltin *et al.*, 1986). Thus, it is possible that there is a negative feedback system, perhaps via Group III and IV afferents, that regulates O_2 delivery to working muscle. Such a feedback system could account for the exponential shape of the $\dot{V}O_2$ response to a work stimulus.

One possible method of determining the effect of delivery on $\dot{V}O_2$ kinetics is to alter inspired O_2. Oxygen uptake kinetics do not change for moderate work at 223, 2,286, and 3,810 m above sea level (Bason *et al.*, 1973). Linnarsson (1974) found no change in the ON kinetic response of $\dot{V}O_2$ measured for various inspired fractions of oxygen (14 %, 21 %, and 30 %) in response to a step change from rest to 200 W. Griffiths *et al.* (1986) also found no difference in $\dot{V}O_2$ tau between 12 and 30 % inspired O_2 for a 60 W ON step. Hogan and Welch (1984) varied the inspired O_2 fraction (16, 21, and 60 %) and exercised subjects at 90 and 95 % $\dot{V}O_2$max. Although their data are somewhat equivocal, it appears that the $\dot{V}O_2$ time course under hypoxic conditions is slightly slower relative to hyperoxia but not compared with normoxia. Xing *et al.* (1991) determined that the phase 2 $\dot{V}O_2$ response was faster in normoxia than in hypoxia (14 %) for a 25-110 W step change. Murphy *et al.* (1989) found a significantly slower $\dot{V}O_2$ time constant in hypoxia (14 %) for step changes from 25 to 105 W compared with normoxia. Linnarsson *et al.* (1974) noted that hypoxia (0.68 ATA) increased and hyperoxia (1.40 ATA) decreased the O_2 deficit for a step increase in work rate from rest to 50 % sea level $\dot{V}O_2$max. Although $\dot{V}O_2$ was not measured continuously, it was concluded that $\dot{V}O_2$ tau averaged 47, 40, and 29 s as the ambient PO_2 increased. The above studies provide somewhat conflicting results, however, studies that used a large number of repetitions or a large step increase in work rate or both, did observe a $\dot{V}O_2$ tau difference between normoxia and the experimental FIO_2 condition. It must be kept in mind that all of the above studies used the same absolute WR for kinetic determination of $\dot{V}O_2$. A constant, absolute work rate used for each condition would be more stressful for a hypoxic condition. This may increase the phase 4 component for the more hypoxic condition, thereby producing a longer estimate of the time constant estimated from a single exponential model.

Raynaud *et al.* (1973) demonstrated that O_2 delivery to exercising muscle (*i.e.*, $\dot{Q}$ · arterial $[O_2]$) has a faster response than time course of $\dot{V}O_2$ measured at the mouth. Additionally, these authors demonstrated that the amount of O_2 returning to the lungs (*i.e.*, $\dot{Q}$ · venous $[O_2]$) is higher during the first 40 s of exercise. These observations suggest that the rate limiting step in

$\dot{V}O_2$ kinetics is O_2 extraction by the working muscle. The indicated early change in venous PO_2 may be caused by vasoconstriction of both the skin and splanchnic areas forcing blood with a slightly higher PO_2 to the venous system. However, the delivery system could have even slower kinetics than the utilisation system and still not be limiting because of the effect of 2 potential buffer systems. First, the a-vO_2 difference may increase. Secondly, the capillary hematocrit may increase. At rest, the capillary hematocrit is much lower than the arterial hematocrit (Duling, 1983; Klitzman and Duling, 1979). Upon initiation of increased contractile activity, vasodilatation causes the capillary hematocrit to rise (Klitzman and Duling, 1979). These two buffer systems affecting O_2 delivery may explain why striated muscle stimulated at a low frequency can increase its O_2 consumption without a concurrent increase in blood flow (Belloni *et al* ., 1979).

The effect of a redistribution of blood flow further indicates that $\dot{T}O_2$ is more important than $\dot{Q}$ in determining the $\dot{V}O_2$ response. Reflex vasoconstriction of numerous vascular beds will divert an increasing proportion of $\dot{Q}$ to the working muscles. In rabbits, approximately 40 % of the increased blood flow to working skeletal and cardiac muscle was attained by vasoconstriction-induced diversion of blood flow (Hales and Ludbrook, 1988). This effect was abolished by arterial barodenervation. Hales and Ludbrook (1988) concluded that the redistribution of blood flow to the working muscles in the rabbit reduces and perhaps abolishes the effect of delivery on $\dot{V}O_2$ kinetics. This effect may be more important in phase 1 than in phase 2 of the $\dot{V}O_2$ response. In human subjects, concurrent measurement of $\dot{Q}$ and femoral artery blood flow indicates that 30 % of increased blood flow to legs performing rhythmic contractions every 2 s may be attributed to redistribution (Waaler *et al.*, 1987). During supine cycle ergometry, is was found that femoral artery blood flow has a similar time constant as $\dot{Q}$ of about 10 s (Eriksen *et al.*, 1990). Moreover, the calculated increase in femoral artery blood flow exceeded that of cardiac output indicating a significant redistribution of blood flow during light supine exercise humans.

Concurrent time course measurements of muscle blood flow and pulmonary $\dot{V}O_2$ kinetics by Ishii *et al.* (1992) demonstrated that the half time response of muscle blood flow was 4-10 fold

faster than the half time of $\dot{V}O_2$. Furthermore, a reduction in muscle temperature affected muscle blood flow and its kinetic response but did not affect $\dot{V}O_2$ kinetics. Although the authors used only one trial to estimate $\dot{V}O_2$ half time, the dramatic difference in kinetic response between blood flow and $\dot{V}O_2$ cannot be ignored.

Another way to alter blood flow to working muscle is by circulatory occlusion. Cuffing the non-exercising limbs is thought to augment blood flow to the exercising limbs. This manipulation is associated with a faster $\dot{V}O_2$ response to a step increase in work rate (Hughson and Inman, 1986a). The time constants for $\dot{V}CO_2$ and $\dot{V}E$ are not affect by this manoeuvre (Hughson and Inman, 1986a).

Clinical studies may also be used to infer the locus of $\dot{V}O_2$ kinetic regulation. A low $\Delta\dot{V}O_2/\Delta WR$ during incremental or ramp exercise is an indication that the working muscles are not adequately supplied with O_2 (Hansen *et al.*, 1987). Patients with a circulatory disorder such as peripheral vascular disease, ST-segment depression, or pulmonary vascular disease frequently exhibit a low $\Delta\dot{V}O_2/\Delta WR$ (Hansen *et al.*, 1987). Thus the rate of O_2 utilisation and the rate of ATP demand must diverge to a greater extent than in healthy subjects. As has been argued in section 5.0, this infers that the $\dot{V}O_2$ kinetic response is slower in patients suffering from circulatory dysfunction. Furthermore, this suggests, albeit without knowledge of the patient's muscle metabolic profile, that the locus of $\dot{V}O_2$ kinetic regulation is central.

Chronic studies of blood flow change during endurance training have rarely been undertaken. Vanderhoof *et al.* (1961) did determine that after training forearm muscles for 28 weeks, the blood flow debt was then less than in the untrained state. The authors speculate that the blood flow deficit must therefore have been less. This implies faster kinetics for blood flow at exercise onset.

6.1.2.3 REGULATION OF $\dot{V}O_2$ KINETICS AT THE CAPILLARY

The muscle capillary bed is a logical site to consider as the locus of $\dot{V}O_2$ kinetic regulation. It is here that O_2 in the blood is unloaded to the cell. If the capillary bed is the locus of $\dot{V}O_2$ kinetic regulation then it should be expected that factors which influence unloading of O_2 to muscle will affect $\dot{V}O_2$ tau. Muscle capillarity is enhanced by training. Since steady state blood flow to a muscle does not change with endurance training, an increase in the capillary to muscle fibre ratio will increase the capillary transit time thereby augmenting O_2 unloading. Additionally, a greater capillary to muscle fibre ratio will reduce the capillary-to-mitochondrion PO_2 gradient required for a given O_2 flux from the capillary to the mitochondria because the radial diffusion distance will be less. Unfortunately, the simultaneous time course of augmented capillarity and a reduced $\dot{V}O_2$ tau induced by training has not been investigated.

Training has been reported to augment the Bohr effect (Braumann *et al.*, 1982). As such, part of the influence of a decreased $\dot{V}O_2$ tau with training may come from the Bohr effect. Training also increases the O_2 tension at 50 % O_2 saturation of Hb as well as increasing the 2,3 diphosphoglyceraldhyde concentration. This effect is explained by the preponderance of young RBCs and the lack of very old RBCs (Mairbaurl *et al.*, 1983). However, acute alteration in the Bohr effect by oral ingestion of ammonium bicarbonate and ammonium chloride to increase and decrease blood pH, respectively, does not affect $\dot{V}O_2$ tau significantly when exercising at a WR equivalent to 75% of the lactate threshold work rate (Oren *et al.*, 1982). Acute alteration of arterial pH also does not affect steady state $\dot{V}O_2$ (Jones *et al.*, 1977).

Much of the discussion on the possible regulation of $\dot{V}O_2$ kinetics by $\dot{Q}$ kinetics assumes or implies that an increase in blood flow to the skeletal muscle capillary bed is similar to the increase in $\dot{Q}$ at exercise onset. This may be far from the truth. When the gracilis muscle was stimulated electrically in the anaesthetized dog, skeletal capillary blood flow tripled within 5 s (Honig *et al.*, 1980). This is an order of magnitude faster than the response of the heart rate. The time constant

for the increase in blood flow to human quadriceps muscle during rhythmic exercise is about 10 s. During this time, average blood flow to the quadriceps increases four fold (Walloe and Wesche, 1988). In addition, the skeletal muscle capillary hematocrit increases during stimulation (Klitzman and Duling, 1979), although the exact time course of this effect has not been reported.

The time course of muscle PO_2 at the onset of stimulation in a dog gracilis muscle preparation is very different from the $\dot{V}O_2$ time course (Connett, 1986). There is actually an initial increase evident within 5 s representing an increase in O_2 delivery to the muscle. This implies that the diffusion of O_2 into the muscle cell is a very rapid process. After the initial muscle PO_2 increase, the PO_2 then decreases till about 30 s then again increases. It appears that convection of O_2 in the capillary occurs at a much faster rate than the $\dot{V}O_2$ response and thus cannot be considered as the mechanism regulating $\dot{V}O_2$ kinetics.

6.1.2.4 INFLUENCE OF O_2 STORAGE CAPACITY ON $\dot{V}O_2$ KINETICS

Different tissues within the body have a differing capacity for O_2 storage. From the point of view of $\dot{V}O_2$ kinetics these loci are both centrally and peripherally located. Alteration in the ability of the body to store O_2 will affect $\dot{V}O_2$ kinetics. It is well known that the body's capacity to store more CO_2 than O_2 is the major factor in the slower CO_2 kinetics observed (Casaburi *et al.*, 1989b; Young and Woolcock, 1978). If the CO_2 storage capacity is increased by volitional hyperventilation prior to exercise, a large increase in $\dot{V}CO_2$ $t_{1/2}$ is observed (43 to 71 s) (Ward *et al.*, 1983). $\dot{V}O_2$ $t_{1/2}$ is also slightly prolonged by this type of perturbation (31 to 39 s). Certainly, under nonsteady state conditions a change in O_2 stores (*e.g.*, working muscle, venous blood, and lung tissue) will affect $\dot{V}O_2$ kinetics (Whipp and Wasserman, 1972). Quantitatively, the contribution of the O_2 stores to the O_2 deficit is quite small (Edwards *et al.*, 1972). When PIO_2 is altered, the O_2 stores change significantly and are positively correlated with $\dot{V}O_2$ tau (Linnarson *et*

al., 1974). Under these conditions however, PIO_2 is probably more important than the size of the O_2 store in regulating $\dot{V}O_2$ tau.

Inman *et al.* (1987) determined that myoglobin stores have little effect on $\dot{V}O_2$ kinetics due to the small volume of O_2 attached to myoglobin and even smaller volume involved during a moderate step increase in work rate. However, this does not imply that myoglobin cannot alter the rate at which O_2 is used by affecting the intracellular PO_2 value. The role of myoglobin in the actual facilitation of O_2 utilisation is controversial. Under hypoxic conditions, myoglobin facilitates O_2 flux. Under normoxic conditions, myoglobin may only have a significant influence on less vascularized tissue such as skeletal muscle rather than cardiac muscle (Wittenberg and Wittenberg, 1989). However, since the $[O_2]$ bound to myoglobin may be 100 times greater than the free $[O_2]$ and yet myoglobin diffusivity is only about 20 fold less than O_2 (Honig *et al.*, 1992) it would appear that myoglobin will be a major contributor to O_2 conductance in most conditions.

Myoglobin may alter the location of the PO_2 gradient between the capillary and the mitochondrion. This can occur by gaseous oxygen binding to myoglobin at the sarcolemma and then diffusing away. This will maintain a steep O_2 gradient across the sarcolemma and a much flatter gradient within the cell sarcoplasm (Honig *et al.*, 1992; Wittenberg and Wittenberg, 1989).

As indicated above for CO_2 kinetics, the greater the buffer capacity of a gas storage system, the slower is the kinetics of that gas. Curiously, red muscle, with its greater capillarity and respiratory enzyme concentration, should have faster kinetics than white muscle. Yet the myoglobin concentration in red muscle is greater than in white muscle. Although, the time course of O_2 utilisation determined with respect to different muscle fibre types is not known, the above suggests that the peripheral store of O_2 may have little affect on $\dot{V}O_2$ kinetics. Of interest, it has been observed that, in humans, myoglobin concentration decreases with training (Terrados *et al.*, 1986).

6.1.3 PERIPHERAL REGULATION OF OXYGEN KINETICS

6.1.3.1 INTRODUCTION

A peripheral regulation site of muscle cell O_2 utilisation ($\dot{Q}O_2$) can be justified conceptually. In a muscle cell, it is the contractile activity that determines the rate of energy utilisation and production. Thus contractile activity determines O_2 consumption. It is not the supply of O_2 to the muscle which regulates ATP production and contractile activity. Intuitively, locating control of O_2 utilisation at the site where the ATP demand occurs is a better design in terms of coupling ATP demand to O_2 utilisation. The Km of O_2 for mitochondrial respiration is well below the O_2 level in muscle tissue indicating an excess of O_2 is present. From this stand-point, it appears that a mechanism located peripheral to its source of supply regulates O_2 utilisation (Gollnick and Hodgson, 1986). Furthermore, in response to electrical stimulation, intracellular PO_2 of a dog gracilis muscle preparation increases above baseline for the first 15 s (Honig *et al.*, 1984: check). If the O_2 supply is limiting then one would expect a higher rate of glycolysis, a higher respiratory exchange ratio, and a lower venous PO_2 value than is commonly observed at rest or at a moderate work rate.

There has been infrequent support for a peripheral locus regulating $\dot{V}O_2$ kinetics when whole body metabolism has been studied. This is due in part to a greater degree of difficulty in conducting such research and perhaps to an omission of reasonable alternative explanations in studies supportive of a central locus for $\dot{V}O_2$ kinetic regulation.

6.1.3.2 THE RELATION OF MUSCLE METABOLISM TO OXYGEN KINETICS DURING EXERCISE

One step in the process of justifying a peripheral locus for $\dot{V}O_2$ kinetic regulation is identification of a rate limiting modulator. Many regulators have been proposed (Balaban, 1990; Mahler, 1980) but it is beyond the scope of this thesis to review oxidative control mechanisms. However, to be more precise about many of the concepts relating muscle metabolism to O_2 regulation, the creatine phosphate shuttle hypothesis will be used to represent a peripheral locus regulator. This hypothesis has been investigated in preparations ranging from mitochondrial fragments to whole body metabolism and the decrease in muscle creatine phosphate concentration is inversely proportional to an increase in the work rate stimulus imposed on the muscle.

The creatine phosphate shuttle hypothesis indicates that the concentration gradient of creatine phosphate (CP) and creatine (C) between the mitochondrion and the myofibrils generates the rate of oxidative flux. The larger the CP and C concentration gradients are, the greater is the oxidative flux. When the oxidative flux increases, the flux of O_2 from the capillary to the mitochondrion must necessarily increase. Thus, the concentration gradients of CP and C determine the O_2 flux into the muscle cell.

During a step increase in work rate, there is an instantaneous decrease in [CP] and increase in [C] at the actin-myosin locus (Fig. 6-1). The increase in [C] at the actin-myosin site will increase the flux of C to the mitochondrion. The increased presentation of C to the mitochondrion will increase the generation of high energy phosphate and hence O_2 utilisation. This will reduce the PO_2 at the mitochondrion resulting in a larger O_2 pressure gradient between the blood and the mitochondrion. This will increase the O_2 flux into the cell. The reduced [CP] at the actin-myosin site will augment shuttling of CP generated at the mitochondrion to the actin-myosin locus. In this

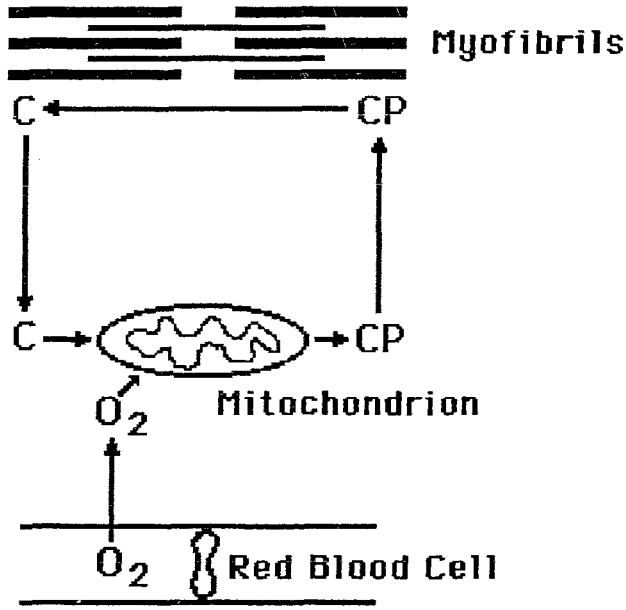

Fig. 6-1. Schematic representation of the creatine phosphate shuttle and O_2 flux into a muscle cell from a capillary.

process, the error signal between the current C, CP, and O_2 concentration gradients and those demanded in the steady state will become less as steady state is approached. The rate of change of each concentration gradient will be proportional to the error signal and thus be exponential in character.

The creatine phosphate shuttle concept permits many predictions. One prediction is that the time course of change in [CP] should be similar to the time course of change in O_2 utilisation. Both NMR studies on humans during exercise (Binzoni *et al.*, 1992; Challiss *et al.*, 1987; Meyer, 1988, 1989; Meyer *et al.*, 1986; Mole *et al.*, 1985) and muscle biopsy studies from electrically stimulated frog muscle (Mahler, 1985), indicate the kinetics of CP reduction at the start of exercise are similar to that of oxidative metabolism. Moreover, Marsh *et al.* (1991) have shown that, for human wrist flexor muscle during ramp exercise, log Pi/CP increases slowly then reaches a threshold at which the ratio increases more rapidly (c.f. Nishida, *et al.*, 1992). This trend is very similar to the slow rise in $\dot{V}O_2$ tau as the step increase in WR increases towards the

lactate threshold WR and a faster rise to step WR changes greater than the lactate threshold WR (Casaburi *et al.*, 1989a).

The existence of a cellular regulator for O_2 consumption implies that the change in the regulator during exercise should be linearly related to the change in O_2 utilisation. Data from stimulated *in vivo* frog muscle (Mahler, 1985), *in situ* rat muscle (Meyer, 1988) and dog muscle (Connett and Honig, 1989; Hogan *et al.*, 1992a; DiPrampero and Margaria, 1968), and exercising human muscle (Linnarsson *et al.*, 1974) have confirmed this. If there is no CP depletion, there is no O_2 flux; similarly if there is 100 % CP depletion there is 100 % or maximal O_2 flux. The linear relation of CP depletion to increasing muscle oxygen consumption ($\dot{Q}O_2$), indicates a 50 % depletion may account for a 50 % O_2 flux. If the capacity of O_2 flux from the capillary to the muscle cell is changed (*e.g.*, by varying FIO_2), the relation of CP depletion to oxygen consumption should not change. A 0 % CP depletion still induces 0 % O_2 flux; a 50 % CP depletion will still induce a 50 % $\dot{Q}O_2max$; and a 100 % CP depletion induces 100 % of the possible O_2 flux. Expressed another way, the maximal concentration and gradient change for C and CP will induce the maximal possible O_2 flux into the muscle cell.

An example of a subject exercising on a cycle ergometer may clarify this concept further. Assume that the subject is working at 100 W and is in a steady state. The changes in the concentration gradient of both C and CP have already been established in order to generate the O_2 flux and the rate of high energy phosphate production demanded by the work rate. If there then is a reduction in FIO_2, this will decrease the O_2 pressure gradient between the blood and the mitochondrion. Thus the O_2 flux into the cell will be reduced. Since the work rate has not changed the demand rate for high energy phosphates has not changed. This reduction of FIO_2 will cause a reduction in [CP] and an increase in [C] similar to that occurring for a step increase in work rate discussed previously. The increase in [C] at the mitochondrial site will increase O_2 utilisation at the mitochondrion resulting in a reduced PO_2 at this locus. The reduced mitochondrial PO_2 increases the pressure gradient of O_2 between the mitochondrion and the blood

thereby compensating for its initial blood PO_2 reduction when FIO_2 was reduced. Only the concentrations of C and CP have changed. There is a greater depletion of CP in the more hypoxic condition. The O_2 flux remains the same in the steady state under the two FIO_2 conditions. Since $\dot{V}O_2max$ is reduced in the hypoxic condition, a greater percentage of potential O_2 flux occurs to meet the energetic requirement and the relation between CP depletion and O_2 flux is maintained.

Prediction of the relation of [CP] to % $\dot{V}O_2max$ has not been specifically investigated. However, there are some incidental data to support this concept. Linnarsson *et al.* (1974) exercised subjects in a barometric chamber at ambient pressures of 0.68, 1.00, and 1.40 ATA. The corresponding maximal O_2 uptake at each pressure was 3.38, 3.91, and 4.34 $l \cdot min^{-1}$, respectively. Submaximal work at 147 W produced corresponding $\dot{V}O_2$ values of 2.12, 2.08, and 2.11 $l \cdot min^{-1}$ respectively. This corresponds to 62, 53, and 49 % of $\dot{V}O_2max$. Phosphagen depletion at $\dot{V}O_2max$ was about 18 $mmoles \cdot kg^{-1}$ for all conditions. Using data from their Fig. 1, estimated phosphagen depletion during submaximal work was about 8.00, 9.75, and 10.75 $mmol \cdot kg^{-1}$ respectively in the 3 conditions. Expressed as a percentage of phosphagen depletion during maximal exercise this is 60, 54, and 44 % respectively (Fig. 6-2). These values are very similar to the % $\dot{V}O_2max$ of the submaximal work rate. More recently, the companion studies of Arthur *et al.* (1992) and Hogan *et al.* (1992a) have presented similar data from an *in situ* dog gastrocnemius muscle preparation confirming the whole body data of Linnarsson *et al.* (1974). This supports the suggestion that when the maximal rate of O_2 flux is altered, a new $\dot{V}O_2$-phosphagen depletion relation is established, and moreover, the linear dependence of $\dot{Q}O_2$ on phosphagen depletion is maintained.

Hogan *et al.* (1992b) studied the dog gastrocnemius preparation and measured a variety of cardiovascular and biochemical parameters under normal, ischemic, and hypoxic conditions at rest and at two different stimulation (not work) rates. These investigators concluded that: "the proposed regulators of tissue respiration did not correlate strongly with O_2 delivery; for example, at very different O_2 deliveries and $\dot{V}O_2$ values, almost identical values of [PCr] were found,

indicating that it was likely that some intracellular factor, possible changes in mean intracellular PO_2, was modulating these regulators" (Hogan *et al.*, 1992b: p1667). Their statement contradicts the aforementioned creatine phosphate shuttle regulation hypothesis yet their data support it. Hogan *et al.* (1992b) found that under hypoxic and ischemic conditions (*i.e.*, when maximal O_2 flux is reduced) it took a greater absolute change in [CP] to generate the same $\dot{V}O_2$. On a relative basis their data indicate that it took a 50 % change in [CP] to generate a 50 % change in $\dot{V}O_2$ between rest and maximum. Furthermore, the delivery of O_2 to the working muscle was the same for the hypoxic and ischemic conditions. They found that changes in a variety of biochemical parameters were not different between the two restricted O_2 delivery conditions. This is exactly what should be expected since the maximal O_2 flux was not different. Thus their data seem to support, rather than contradict, the creatine phosphate shuttle hypothesis. Hogan *et al.* (1992b) may have been more correct in saying that although the absolute change in proposed metabolic regulators did not correlate with O_2 delivery, the percent change in proposed regulators was consistent with % $\dot{V}O_2$max observed.

Some investigators have concluded that gross $\dot{V}O_2$ kinetics are regulated centrally by O_2 delivery because a change in FIO_2 causes an inverse change in $\dot{V}O_2$ tau (Hughson, 1990; Linnarsson *et al.*, 1974). However, this does not disprove a hypothesis of $\dot{V}O_2$ regulation at a peripheral or cellular level. When FIO_2 is reduced, a given absolute $\dot{V}O_2$ demand will require a larger O_2 gradient to be established between the vascular compartment and the muscle. This will take longer to establish and thus the rate of increase in muscle O_2 utilisation will be slower. From a "peripheral" perspective, the change in FIO_2 presets the level to which mitochondrial PO_2 has to be reduced in order to establish an adequate O_2 gradient, and thus O_2 flux, from the blood to mitochondrion for the work rate demanded. The change in $\dot{V}O_2$ tau observed when FIO_2 is altered may therefore be explained satisfactorily by either the central or peripheral hypothesis of O_2 regulation.

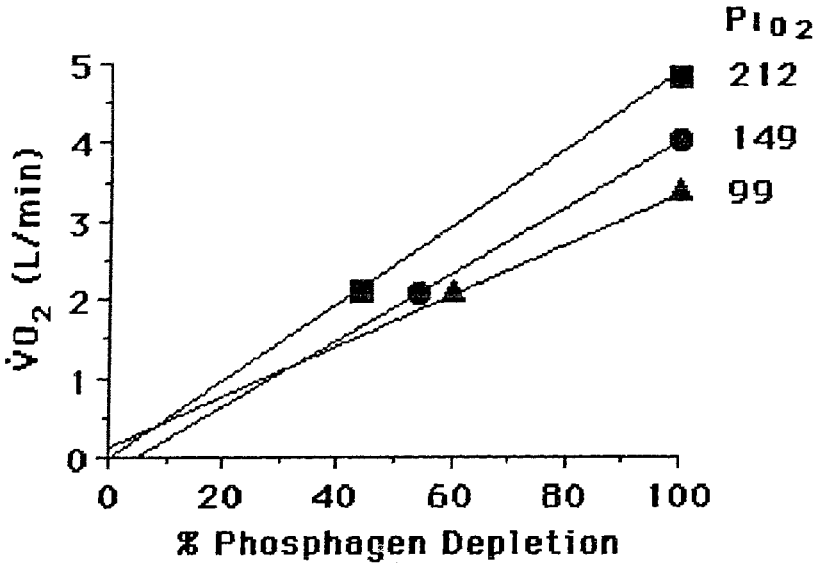

Fig. 6-2. The relation of $\dot{V}O_2$ with percent phosphagen depletion. PIO_2 units are mmHg. Data are recalculated from Linnarsson *et al.* (1974).

When FIO_2 is reduced, then $\dot{V}O_2max$ is reduced and the mitochondrion will require a greater stimulus to reduce mitochondrial PO_2. If this stimulus is creatine or ADP for example, then glycolysis will also be activated to a greater degree. The lactate threshold, measured against absolute $\dot{V}O_2$, is reduced with hypoxia. However, on a relative scale (percent $\dot{V}O_2max$), hypoxia does not change the lactate threshold (Yoshida *et al.*, 1987). One implication of this is that the lactate threshold (implying cellular metabolism) is related more to the percent change in the cellular modulator of $\dot{Q}O_2$ rather than to an absolute change in the modulator of O_2 utilisation.

Evidence presented in this section so far indicates a strong relation between a change in a regulator of O_2 utilisation such as creatine and the rate of O_2 utilisation. There is an equally strong relation between a regulator of O_2 utilisation and the PO_2 of muscle or arterial blood. In human subjects performing foot ergometry, the relation of [CP] with intramuscular PO_2 is linear throughout a range from rest to fatigue (Bylund-Fellenuis *et al.*, 1981). For the same work rate and $\dot{V}O_2$, when arterial PO2 is reduced (decreased FiO2), there is an increase in [NADH] and [C] (Katz and Sahlin, 1987). As O_2 delivery (and arterial PO_2) to a hindlimb preparation is reduced

there is a concomitant reduction in muscle [CP]/[Pi] at rest and during exercise (Gutierrez *et al.*, 1989; Idstrom *et al.*, 1985; Idstrom *et al.*, 1986).

Ramp WR protocols and peripheral regulation

Sahlin *et al.* (1988) examined the size of the O_2 deficit when a steady state work rate was achieved from a step or a slow ramp increase in work rate. One hypothesized outcome of the experiment was that if the rate of increase in work rate is small then only the fast metabolic component of the O_2 deficit (phosphagen depletion) would occur. Thus there would be no additional slow component of the O_2 deficit (glycolysis). Therefore the O_2 deficit induced by an increase in work rate would be smaller if the total step increment was approached by series of small steps rather than by one large step. However, Sahlin *et al.* (1988) found the O_2 deficit to be the same which implied that the time course of the $\dot{V}O_2$ response is not limited by a slow cardiorespiratory response.

Muscle biopsies taken in this study (Sahlin *et al.*, 1988) during steady state indicate no difference in potential respiratory modulators such as CP, C, ATP, ADP, AMP, IMP, NADH, NAD^+, and ΔPi between the two work rate protocols. This is in accord with the concept of a creatine phosphate shuttle regulation because a given change in the [CP] and [C] gradients between the myofibrils and the mitochondrion is necessary to generate a given $\dot{V}O_2$ regardless of the previous work rate protocol in order to attain steady state.

In total, such data indicate there is no repayment of the O_2 deficit during exercise. Furthermore, the coupling of anaerobic to aerobic energy supply is very robust and is not modified by hypothetical constructs of independent fast and slow O_2 deficit components.

Recently, the utility of the ramp work rate protocol to define $\dot{V}O_2$ kinetic parameters has been criticized because of apparent non-linearity of the $\dot{V}O_2$ response and it inability to discriminate the hypoxic response from the normoxic response. Murphy *et al.* (1989) determined that, when breathing hypoxic air, the total lag time (TLT: the sum of $\dot{V}O_2$ tau and the time delay) is not different from normoxic conditions during a ramp but it is prolonged during a step. Because of

this, the authors criticized the ramp work rate protocol as an insensitive method even though, concomitantly, the $\Delta\dot{V}O_2/\Delta WR$ response was less during hypoxia. The similar TLT and different $\Delta\dot{V}O_2/\Delta WR$ between the two conditions can be predicted from the above mentioned model. As already noted for a step work rate, $\dot{V}O_2$ tau increases with an increase in the size of the step and is thus work rate dependent. However, the equation used in the literature to describe the $\dot{V}O_2$ response to ramp work has an invariable time constant despite a changing work rate (see section 5: Fig. 5-1). This seems inconsistent. An alternative is substitution of a work rate dependent time constant in the equation describing the $\dot{V}O_2$ response (see section 5: Fig. 5-2) to ensure an increasing divergence of the $\dot{V}O_2$ response from the accompanying ramp work rate demand. It is interesting to note that Murphy *et al.* (1989) found a diverging $\dot{V}O_2$ response but a similar TLT to a 40 $W \cdot min^{-1}$ ramp stimulus during hypoxic conditions compared with normoxic conditions. The TLT was determined from a projection of the $\dot{V}O_2$ response back to baseline. In this case the baseline was 25 W. A discernible difference in $\dot{V}O_2$ tau would not be expected for such a small work rate as 25 W.

The diverging slope of the hypoxic $\dot{V}O_2$ response from the same ramp work rate stimulus suggests that the time constant of the $\dot{V}O_2$ response is increasing at least for the hypoxic condition. It is quite logical to expect a similar effect, though less discernable, of the $\dot{V}O_2$ response under normoxic conditions if such data are analysed appropriately. $\dot{V}O_2$ tau is longer in hypoxia because the relative WR is always greater than in normoxia. This requires a larger energy modulator gradient to be established and more time will be needed to established this gradient. This latter model predicts a longer $\dot{V}O_2$ tau for a step increase in work rate and a $\dot{V}O_2$ response that diverges from the incremental energy demand of a ramp work rate stimulus under hypoxic conditions. Using this model, a ramp protocol would be a sensitive discriminator of the hypoxic condition.

Training effects and peripheral oxygen uptake regulation

Any explanation of a training-induced faster $\dot{V}O_2$ response from a peripheral perspective, regardless of the metabolic hypothesis that accounts for cellular O_2 regulation, will almost certainly incorporate a contribution made by an increase in oxidative enzyme activity which also occurs with training.

Holloszy (1967) was the first to document a training-induced increase in oxidative enzyme activity. Holloszy also indicated that enhanced oxidative activity would enable a given submaximal $\dot{V}O_2$ to be maintained with a lower concentration of potential O_2 regulators such as ADP and Pi (Holloszy 1967; 1976). Gollnick and Saltin (1982) elaborated on this concept to propose that an altered energy metabolism induced by training was due to an enhanced metabolic sensitivity that occurs in pathways which have a training-induced increase in enzyme activity. These authors indicate that with an augmented oxidative capacity after endurance training, a given flux through the oxidative pathways can be maintained with a smaller change in substrate concentration. As early as 1972, it was known that muscle metabolites change less in response to a given work rate after training (Karlsson *et al.*, 1972). However, it was not known if these changes were due to an enhanced delivery of O_2 or to a greater cellular oxidative capacity. Constable *et al.* (1987) and Dudley *et al.* (1987) were the first to demonstrate directly that when mitochondrial content is increased by training, a given oxidative rate can be maintained by a smaller change in regulatory metabolites independent of O_2 delivery. A strong relation has been shown when the time constant of O_2 kinetics is compared to blood lactate concentration (Cerretelli *et al.*, 1979; Yoshida *et al.*, 1992).

Rather than perceive the faster training-induced O_2 kinetics as a result of a greater activity of enzyme, for *in vivo* conditions it may be more appropriate to view faster O_2 kinetics after training as a reduction in diffusion distance. The end effect is similar; instead of a lower substrate concentration maintaining a given flux with a higher enzyme activity, a lower substrate concentration maintains a given flux because the diffusion distance is less. The increase in

mitochondrial content with training will reduce both the diffusion distance between the blood and the mitochondrion and between the myofibrils and the mitochondrion. Unfortunately, the time course of mitochondrial changes has not been compared to the $\dot{V}O_2$ kinetic change induced by training.

6.1.3.3 GLYCOLYTIC POWER

Substrates entering the TCA cycle come from 2 major sources: glycolysis and beta-oxidation. It is possible that $\dot{V}O_2$ kinetics are different for the two different fuel sources. If there was no carbohydrate oxidation present then $\dot{V}O_2$ tau would depend only on the lipid source. Without flux through the glycolytic pathway there would be a major reduction in the size of the O_2 deficit. If the O_2 deficit is reduced then $\dot{V}O_2$ tau must be shorter. This is implicit in a rearrangement of equation 5.

$$\tau = O_2 \text{ deficit} / \Delta\dot{V}O_2(ss) \quad \text{.....(6-3)}$$

The slow increase in log Pi/CP during ramp work up to lactate threshold with a faster increase thereafter (Marsh *et al.*, 1991) is very similar to the slow increase in $\dot{V}O_2$ tau at work rates below the lactate threshold and its faster increase above the threshold (Casaburi *et al.*, 1989a). Coincident with the threshold increase in log Pi/CP is a threshold decrease in muscle pH (Marsh *et al.*, 1991). The increased acidosis suggests an augmented glycolytic flux, therefore establishing a relation between glycolysis and $\dot{V}O_2$ tau.

DiPrampero *et al.* (1989) noted as an undocumented observation that $\dot{V}O_2$ kinetics were faster in subjects who had McArdle's disease than in normal adults. Children also have low glycolytic power (Eriksson *et al.*, 1971) and a faster $\dot{V}O_2$ response than do adults (Armon *et al.*, 1991). A possible explanation for a faster $\dot{V}O_2$ response when glycolytic activity is reduced is that glycolysis and respiration compete for the same substrate. This substrate could be ADP or creatine. Whatever the competing substrate, the relative degree of anaerobic glycolytic power to

mitochondrial oxidative power may determine the time-dependent rate of O_2 utilisation. When glycolytic power is reduced, such as when a muscle is glycogen-depleted, the changes in ADP, creatine, and CP may be less for a given submaximal work rate. This may occur because it will take a smaller concentration gradient to generate a given flux between the myofibrils and the mitochondrion when competition for substrate required by the mitochondrion is reduced.

This concept can explain the training-induced decrease in $\dot{V}O_2$ tau. With endurance training, mitochondrial oxidative power within the muscle is increased whereas glycolytic power is largely unchanged. After training, the larger relative mitochondrial oxidative power will enable respiration to compete with glycolysis more successfully for substrate. The faster $\dot{V}O_2$ response in the trained state will be due to a reduced diffusion distance for mitochondrial substrates. As such, a reduction in the gradient for generating a flux of mitochondrial substrates would be anticipated and does occur (Constable *et al.*, 1987; Dudley *et al.*, 1987; Holloszy, 1976). This can explain the significant relation found between the time course $\dot{V}O_2$ tau and the lactate threshold during training (r=-0.76) (Yoshida and Udo, 1992).

The reduced respiratory exchange ratio after training may also be explained by this concept. As mentioned previously, after training the mitochondrion can compete more successfully with glycolysis for common substrate. Since glycolysis will receive less substrate, there will be a reduced glycolytic flux. As such, more substrate entering the TCA cycle will come from beta-oxidation of free fatty acids. A reduced carbohydrate utilisation and an enhanced lipid utilisation will result in a lower respiratory exchange ratio.

Prior exercise can expedite the $\dot{V}O_2$ time course to a subsequent step increase in work rate to above the lactate threshold. However, the prior exercise must also be above the lactate threshold (Gausche *et al.*, 1989; Gerbino *et al.*, 1990). One possible explanation for this phenomenon is that for the subsequent exercise, there is a higher muscle lactate concentration present when the prior exercise is above the lactate threshold. The higher muscle lactate concentration will reduce the requirement for glycolysis, thus the mitochondrion will be able to compete more successfully

for common substrate. The end result is a faster $\dot{V}O_2$ response and a smaller O_2 deficit. A similar effect is observed in an isolated heart preparation. When the perfusate contains a high glucose concentration, the mitochondrial oxygen utilisation response time is 20 % slower (11.9 s) than when glucose is replaced with pyruvate (10.0 s) (Hak *et al.*, 1993).

6.1.3.4 ENZYME RECRUITMENT

Recruitment is a common strategy by which the body regulates power output. If more power is required, more muscle units are recruited to meet the demand. Once recruited, a muscle unit can be activated to a varying degree. The stronger the activation, the greater the transient increase in cytosolic [Ca^{2+}]. A small increase in cytosolic [Ca^{2+}] will bind to only a few troponin sites and thus expose only a few actin sites for ATP hydrolysis. A larger increase in cytosolic [Ca^{2+}] will result in a greater number of sites exposed to ATP hydrolysis. In essence the degree of cytosolic [Ca^{2+}] increase determines how many ATPase sites will be recruited for muscular contraction. Thus the Ca^{2+} signal determines the rate of energy utilisation.

Calcium also regulates energy production thereby integrating cellular energy homeostasis. A well known example of Ca^{2+} stimulating energy production is its activating effect on certain mitochondrial dehydrogenases (see Hansford (1985) for a review). More recently, it has been suggested that ATP synthase may be up-regulated to a more active state rather than always existing in active form (see Harris and Das (1991) for a review). Ca^{2+} has also been shown to stimulate the up-regulation of ATP synthase indirectly, perhaps via it effects on ATP synthase inhibitory proteins. Thus Ca^{2+} again shows a recruiting effect: this time on energy synthesis.

The recruitment of ATP synthase has been used to explain how myocardial energy production can increase without a change in ADP or Pi. This is because substance flux can be increased by increasing the surface area independently of substance concentration. If the rate of energy production increases, without an increase in ATP synthase substrate concentrations, this implies

that time constant for the recruitment of ATPase synthase is less than frequency of sampling. A common sampling frequency for NMR is about one per 15 s.

In cultured cardiomyocytes, the time course of ATP synthase recruitment (time constant ~50 s) (Das and Harris, 1989) is similar to the time course of whole body $\dot{V}O_2$ kinetics. This congruence of time courses suggest that ATP synthase recruitment may regulate O_2 utilisation. However, under more *in vivo* like cardiac conditions of an isolated heart preparation, the time constant of myocardial O_2 utilisation has been shown to be 5-10 fold faster (van Beek and Westerhof, 1990; 1991). Therefore, while the ATP synthase recruitment strategy is an appealing regulator of O_2 utilisation from a variety of cellular homeostasis perspectives, the limited data on its time course of recruitment currently does not justify its serious consideration as a regulator of $\dot{V}O_2$ kinetics.

6.2 EXPERIMENT 3-ONE-LEGGED TRAINING AND $\dot{V}O_2$ TAU

Introduction

A multitude of steps are involved at the pulmonary, cardiovascular, and tissue level in the body's utilisation of O_2. Despite this complexity, the unsteady state time course of O_2 utilisation during a step increase in work rate resembles first order exponential kinetics. This indicates that perhaps only one step in the O_2 utilisation process is rate limiting. Controversy arises as to the location of this rate limiting step. Some investigators suggest the rate limiting step is the delivery of O_2 to the working muscle (Hughson, 1990; Linnarsson *et al.*, 1974) while others indicate that the critical step resides within the muscular compartment (Sahlin *et al.*, 1988; Whip and Mahler, 1980).

The controversy whether O_2 utilisation is limited by a central or peripheral process is analogous to a previous, and still equivocal, controversy in cardiorespiratory research: is $\dot{V}O_2$max

limited by O_2 delivery to the muscle or is it limited by a peripheral process located in the working muscle? One approach to elucidate the locus of $\dot{V}O_2$max limitation has been to use one-legged cycling. It was found that $\dot{V}O_2$max increased in the trained leg and, albeit less, also in the untrained leg (Saltin *et al.*, 1976).

It is the purpose of this study to use a one-legged cycling technique to determine if $\dot{V}O_2$ kinetics are faster in the untrained leg after training the contralateral leg.

Methods

Five healthy male subjects volunteered for participation. Their physical characteristics are listed in Table 6-1.

Prior to training, each subject performed slow ramp (1 W per 6 s) cycle ergometry to exhaustion with the leg to be trained. This was used to determine the ventilatory threshold of the leg to be trained. During one-legged cycle ergometer, a 2.5 kg counter weight was added to the free pedal to augment the return of the exercising leg to the upright position.

On subsequent days each subject performed 3 series of 5 step increases between 20 W and the work rate corresponding to 10 W below the single-leg ventilatory threshold of the subject in order to determine the $\dot{V}O_2$ kinetics. The work rate was alternated at 4 min intervals. This was done for each leg. Most often one day separated a single 5 step work rate protocol. Occasionally two 5 step protocols where performed on the same day with opposite legs. When this occurred, at least 3 hours separated each test.

Training took place during a period of 4 weeks. The training was done 4 days per week for 30 min per session. The initial training work rate was the ventilatory threshold work rate. This was incremented each time the subject was able to maintain the work rate for 30 min.

Following 4 weeks of training, the $\dot{V}O_2$ kinetics of each leg were again determined by performing the same 3 series of 5 step work rate increases as done prior to training.

TABLE 6-1. *Characteristics of subjects.*

Subject	Age	Height (cm)	Weight (kg)
1	39	186	86
2	29	172	70
3	30	179	78
4	36	178	70
5	25	186	80

EMG data were collected on one subject to determine how much the untrained leg musculature was activated by the work protocol. The EMG data were collected and processed as described in section 5.

Data Analysis

For each leg, the $\dot{V}O_2$ and HR time constants were determined for pre and post training. The data were interpolated every second, superpositioned, and the median value was selected. Because HR displayed an increasing linear trend from the first to last step, each of the 15 steps for HR were normalized prior to ensembling. The phase 2 and 3 portions of the data were iteratively modeled with a one component exponential model to determine the time constant. The start of phase 2 was usually clearly visible. If it could not be discerned visually, the point of increase of F_ETO_2 was used.

Statistical analysis

The $\dot{V}O_2$ and HR time constants derived from the modeling for pre and post kinetic tests were analyzed in an overall 3-way ANOVA (2 variable factors ($\dot{V}O_2$ and HR), 2 training factors (pre and post), and 2 leg factors (trained and untrained)). The $\dot{V}O_2$ and HR time constants were then

analyzed further with a 2-way ANOVA (2 factors of training (pre and post) and 2 factors of legs (trained and untrained)). Individual preplanned comparisons were done with a t-test. One-tailed probabilities are reported.

Supplemental experiment

After the first subject had completed the pretest exercise regimes, these data were examined. The $\dot{V}O_2$ and HR responses were as expected. Interestingly, the $FET O_2$ data displayed a very clean saturating exponential. These data were then analyzed throughout the experiment similar to the $\dot{V}O_2$ data. The $FET O_2$ change to step exercise has not been examined in detail. $FET O_2$ values are dependent on the ventilatory rate and on the venous PO_2. In order to examine the $FET O_2$ changes in more detail, the ventilatory rate and breathing rate were held constant at 55 $l \cdot min^{-1}$ and 20 $breaths \cdot min^{-1}$ for one-legged cycling. The WR protocol was the same 5 on steps between 20 and 80 W that were used in the one-legged training study. The ventilatory rate was about 10 $l \cdot min^{-1}$ greater than required for the exercise. This was done by the first subject after he had completed the post tests. The same subject also performed the one-legged ramp exercise to exhaustion as in the pre test. However, during this test the ventilatory rate was maintained at 120 $l \cdot min^{-1}$ and breathing rate at 40$\cdot min^{-1}$ for the entire test. In both constant hyperventilatory tests, ventilation was maintained constant by having the subject observe on the Mac II fx computer screen the real time ventilatory rate of each breath.

Results

The overall 3-way ANOVA indicated a significant training x leg interaction ($F = 4.35$, $p = 0.05$). This indicates the trained and untrained leg responded differently to training (Fig. 6-3). A nonsignificant interaction of variable x leg x training ($F = 2.11$, $p = 0.11$) demonstrated that the $\dot{V}O_2$ and HR time constants varied similarly across legs and training.

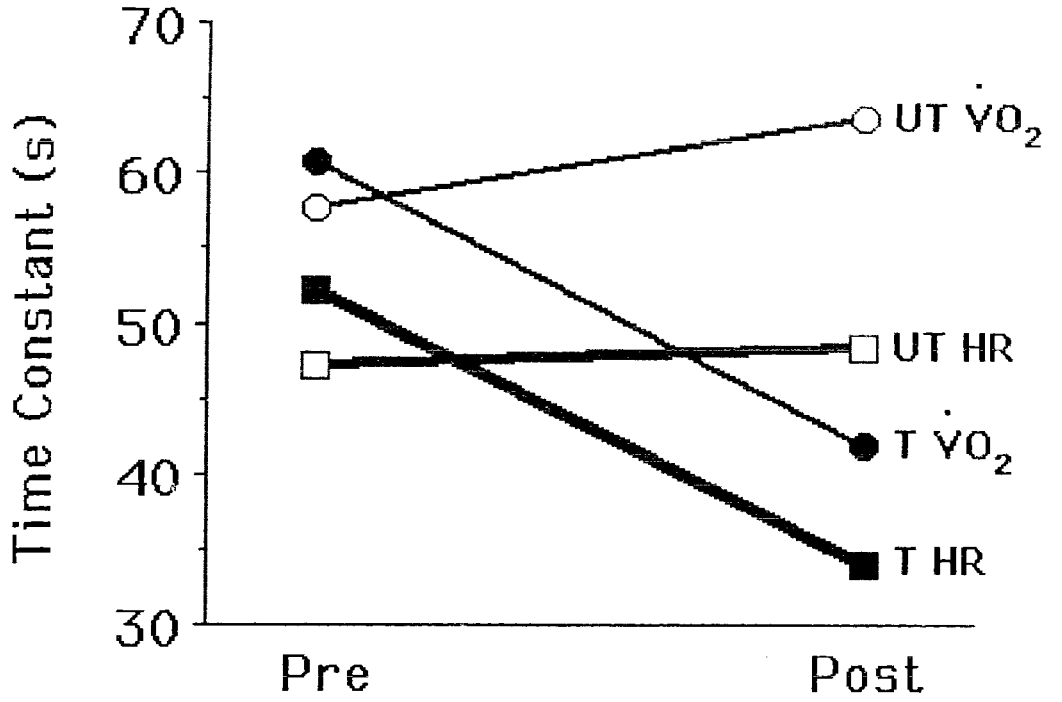

Fig. 6-3. The group mean time constants of $\dot{V}O_2$ and HR for pre and post training. UT = untrained leg. T = trained leg. The $\dot{V}O_2$ SE is approximately 15 % of the value and the HR SE is approximately 10 % of the response value. In this figure, the lines merely give direction to the points pre and post and do not connote any continuity in time course.

There was a significant interaction effect between training and legs for the $\dot{V}O_2$ time constant ($F = 7.94$, $p = 0.024$) indicating that the trained and untrained legs were affected differently by the training protocol. There was no significant interaction for the heart rate time constant ($F = 2.42$, $p = 0.097$). Although all subjects had a reduced HR tau for the trained leg, 3 subjects had a reduced HR time constant in their untrained leg and the other 2 subjects had an increase in the time constant.

The pre and post $\dot{V}O_2$ time constants were also compared by considering the variation of the breath-by-breath $\dot{V}O_2$ data and the $\Delta\dot{V}O_2(ss)$ for the step change in work rate and then determining how great a difference between time constants was required for a certain level of significance as suggested by Lamarra *et al.* (1987) (Table 6-2). Such an analysis indicated that, for each subject, the $\dot{V}O_2$ time constant was significantly shorter ($p<0.05$) for the trained leg after training.

TABLE 6-2. *The oxygen uptake time constant (s) for the trained and untrained legs before and after training and the 95 % confidence interval (s) for a difference between time constants.*

Subject	Trained		Untrained		Confidence Interval
	Pre	Post	Pre	Post	
1	75.0	54.2	74.3	82.8	3
2	48.2	32.0	66.8	61.0	6
3	48.4	32.4	42.4	38.8	5
4	43.4	27.2	39.4	37.6	4
5	89.0	63.2	66.4	89.2	4

Examples of the $\dot{V}O_2$ and heart rate responses to the ensembling and median procedures are presented in Fig. 6-4 and Fig. 6-5.

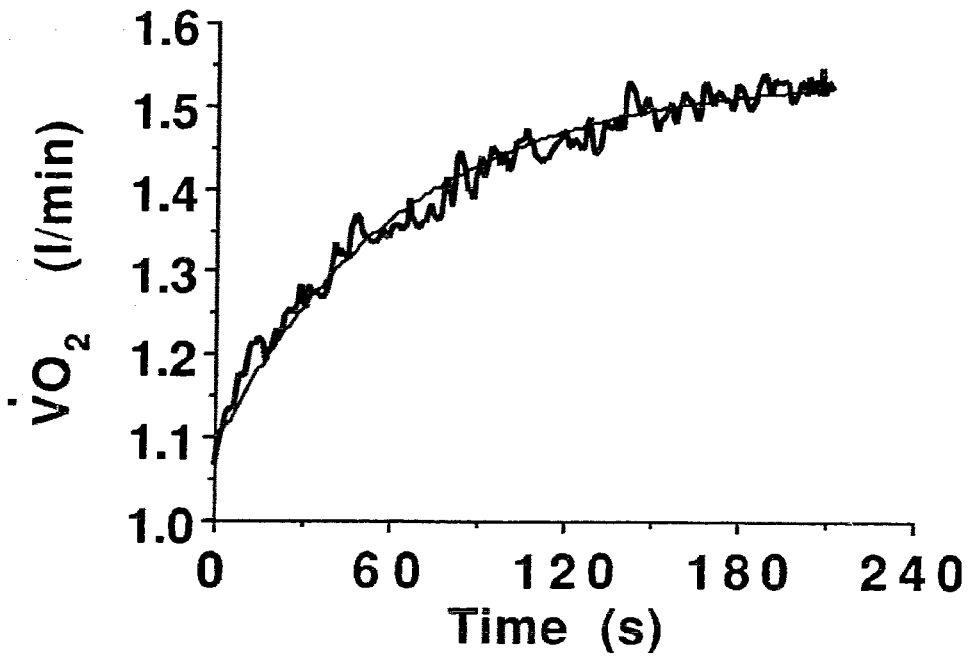

Fig. 6-4. The resultant $\dot{V}O_2$ response (thick line) after taking the median value of 15 time aligned step increases. A single exponential is curve fitted to these data (thin line). The phase one response was removed from these data.

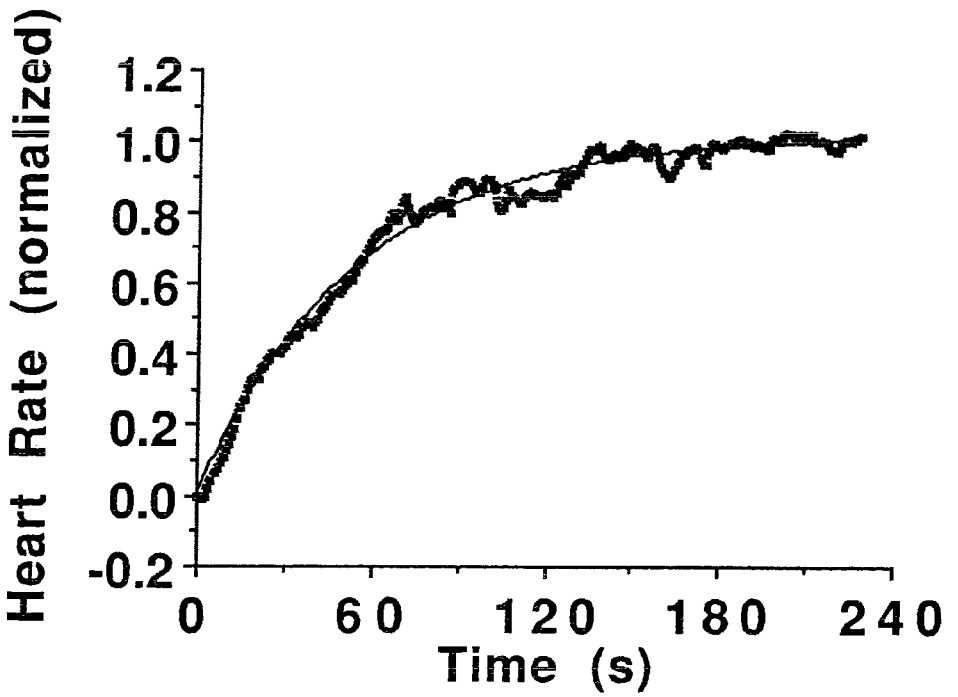

Fig. 6-5. The resultant normalized heart rate response (points) after taking the median value of 15 time aligned step increases. A single exponential is curve fitted to these data (thin line). The phase one response was not removed from these data.

TABLE 6-3. *The high work rate steady state data for oxygen uptake and heart rate.*

	PreT	PostT	PreUT	PostUT
$\dot{V}O_2$ ($l \cdot min^{-1}$)	1.73[a]	1.59	1.71[a]	1.63
S.D.	0.20	0.18	0.21	0.21
HR ($beats \cdot min^{-1}$)	140[a]	128[b]	139	134
S.D.	17	16	15	17

T = Trained leg: UT = Untrained leg.

[a] significantly different ($p < 0.05$) from post training, same leg.

[b] significantly different ($p < 0.05$) from other leg, same training.

The steady state values for $\dot{V}O_2$ and HR were determined from the last 6 data points at each high work rate step. With N = ~75 small differences can be extracted. The $\dot{V}O_2$ was significantly lower during post training for each leg. For the trained leg, after training the heart rate was 12 beats·min^{-1} lower compared with the trained leg prior to training and 6 beats·min^{-1} lower compared with the untrained leg after training.

TABLE 6-4. *Summary of EMG data from one subject while working one leg at 80 W while the other leg was resting.*

	IEMG (mV·s)	MedPF (Hz)	ZC
Working Leg	31.0	70.1	91
Unused Leg	3.1	5.5	5

IEMG = integrated electromyogram: MedPF = median power frequency: ZC = zero crossings.

The effect of the work protocol on the unused leg was minimal by itself and when compared with the working leg (Table 6-4).

Supplemental experiment

In 2 subjects, the small step increase work rate had little effect on ventilation. In such an instance F_ETO_2 values (these values are inverted with room air being zero) displayed an exponential increase with a high signal-to-noise ratio and a characteristic delay before increasing (Fig. 6-6 and Fig. 6-7).

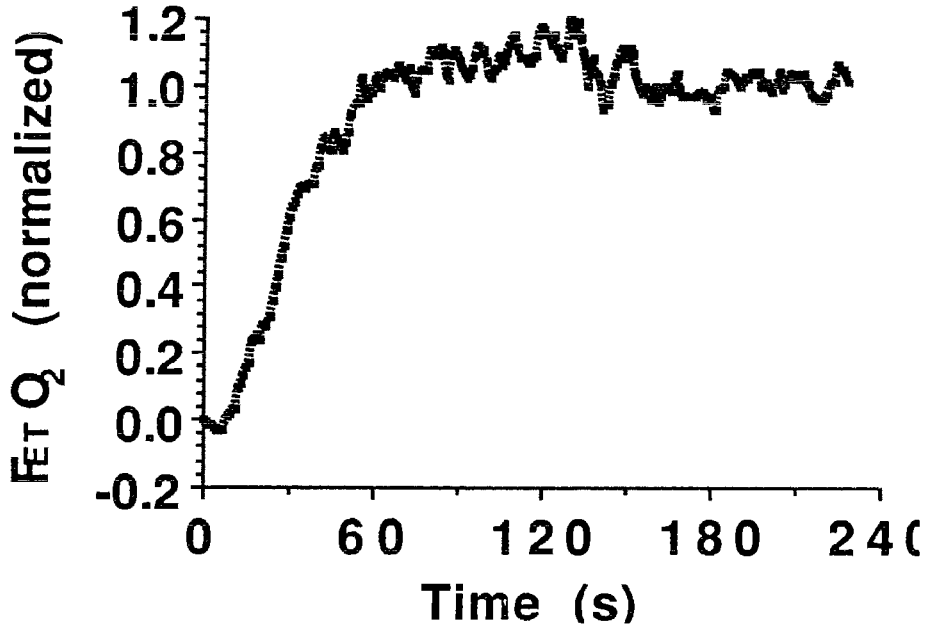

Fig. 6-6. The resultant normalized inverted $F_{ET}O_2$ response (points) after taking the median value of 15 time aligned step increases. The phase one response was not removed from these data.

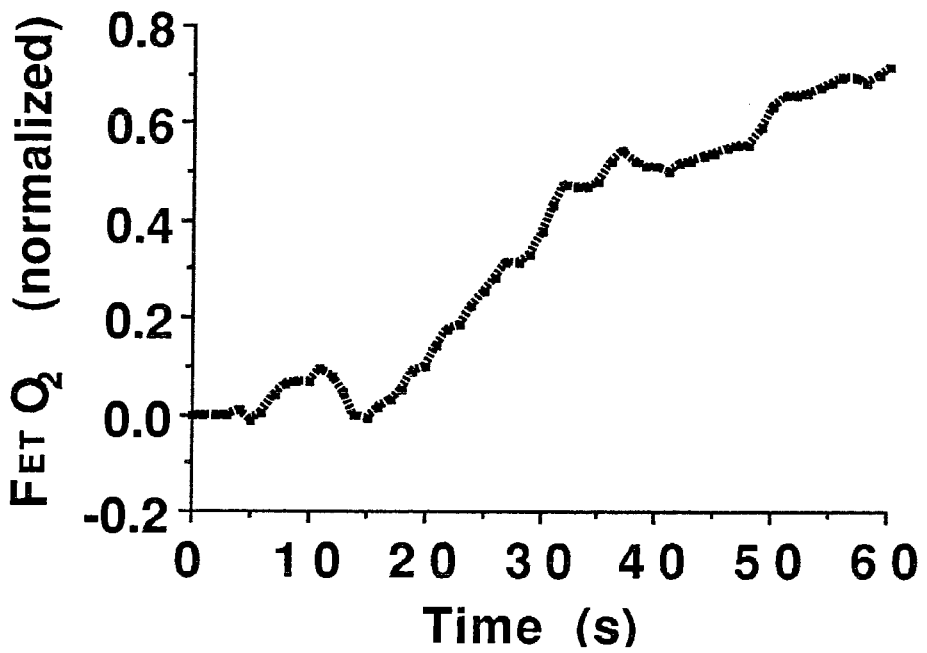

Fig. 6-7. The resultant normalized inverted $F_{ET}O_2$ response (points) after taking the median value of 5 time aligned step increases when ventilation was held constant at 55 $l \cdot min^{-1}$. The phase one response was not removed from these data to emphasize the characteristic time delay of the response.

The $F_{ET}O_2$ response to the one-legged ramp exercise was markedly different for volitional and constant (120 l·min^{-1}) ventilation (Fig. 6-8). With constant ventilation, the $F_{ET}O_2$ response is very linear throughout the increasing work rate stage and did not show any dramatic change in the middle portion of the ramp as with the volitional paradigm. The time to exhaustion was about 30 s less during constant ventilation, probably due in part to respiratory fatigue.

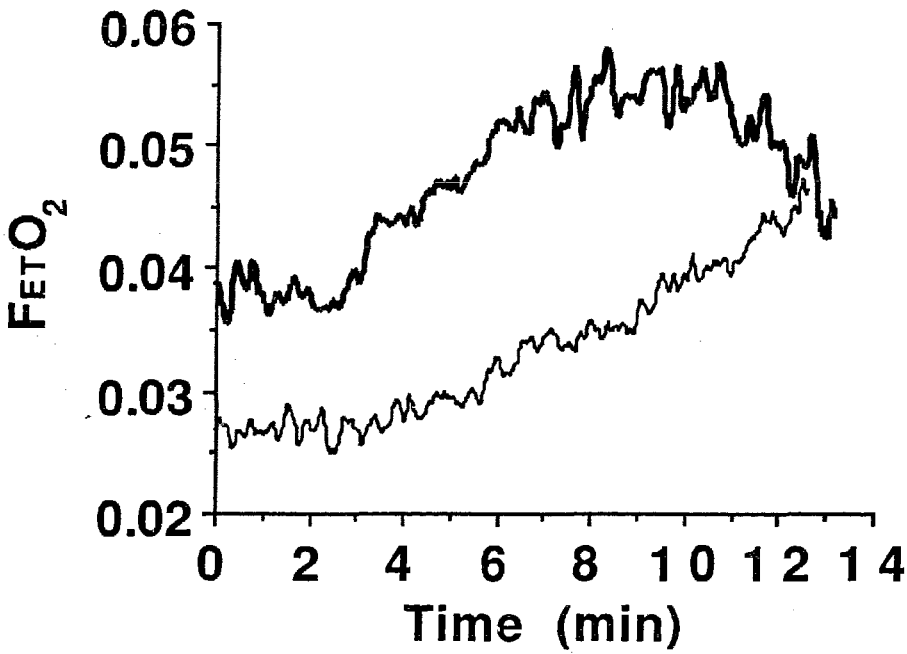

Fig. 6-8. The inverted and smoothed $F_{ET}O_2$ responses to two identical ramp work rate protocols. The ramp onset is at 2 min. For one ramp the ventilation was volitional (thick line) and for the other ramp the ventilation was maintained at about 120 l·min^{-1} throughout. The fractional increase in $F_{ET}O_2$ from 0.03 to 0.05 represents a decrease in alveolar end-tidal PO_2 from 128 to 114 mm Hg.

Discussion

Time constant for oxygen uptake

One-legged training induced a faster $\dot{V}O_2$ response in the trained leg after training compared with the untrained leg. A simple explanation is that $\dot{V}O_2$ kinetics must be regulated peripherally because the delivery system is common to each leg. However the HR response was not consistent between the trained and untrained legs. Saltin *et al.* (1976) noted that submaximal steady state HR differed between trained and untrained legs at the same absolute work rate. This asymmetric HR response between the trained and untrained leg indicates that the delivery responses may be different between the respective legs. This suggests that the delivery process itself may be influenced by peripheral factors. A possible mechanism to explain the above effects may be the well known increase in capilliarity induced by aerobic training (Saltin and Gollnick, 1983). The increased capillarity in the trained leg would reduced vascular resistance. As such, blood flow to the working muscle may increase more rapidly in the unsteady state. Oxygen diffusion distances would also be reduced with the enhanced capillarity thus enabling faster acceleration of oxygen utilisation. Augmented mitochondrial density in the trained leg would have the same effect on oxygen diffusion.

To suggest that oxygen kinetics are regulated exclusively either by a central or peripheral mechanism is an oversimplification. In one extreme, if blood flow did not increase at all, an increase in $\dot{V}O_2$ could be accomplished only by increasing the a-vO_2 difference. This has been examined experimentally. Van Beek and Westerhof (1990; 1991) measured $\dot{V}O_2$ in an *in situ* dog heart preparation. By increasing the heart rate without changing the blood flow, the entire $\dot{V}O_2$ response is met by an increase in the a-vO_2 difference. Van Beek and Westerhof (1990; 1991) demonstrate that the time course of venous PO_2 directly reflects the mitochondrial O_2 utilisation response under these conditions.

In the other extreme, blood flow could increase to such an extent that an increase in $\dot{V}O_2$ could be met without any change in the a-vO_2 difference. The increase in blood flow will increase capillary PO_2. The tissue PO_2 will not need to change because the PO_2 gradient into the tissue and O_2 flux has increased sufficiently. In such a condition, potential phosphagen regulators may not change because the mitochondrial PO_2 does not need to be reduced. In an *in vivo* dog heart preparation, Heineman and Balaban (1990) found that when blood supply is allowed to increase during cardiac pacing, phosphate metabolites do not change whereas they do change predictably if blood supply is reduced under the same conditions. That metabolic regulators such as the phosphagens do not change in some circumstances does not imply they do not regulate O_2 utilisation. If O_2 delivery to skeletal muscle is reduced at rest, there is an increase in [C] even with no change in work or $\dot{V}O_2$ (Hogan *et al.* 1992b). In this situation, phosphagen regulators may still be controlling O_2 utilisation. The overall equation for oxidative phosphorylation is

$$3\ ADP + 3\ Pi + 1/2\ O_2 + NADH + H^+ \longrightarrow 3\ ATP + NAD^+ + H_2O$$

If mitochondrial O_2 is reduced in order to maintain the PO_2 gradient, then another reactant has to increase in order to maintain the same $\dot{V}O_2$. This has been shown in both tissue cultures (Wilson *et al.*, 1979) and stimulated skeletal muscle (Hogan *et al.*, 1992a).

A decrease in arterial PO_2 or O_2 delivery will increase [ADP], [Pi], [NADH], and [H^+] and decrease tissue PO_2 relative to normoxia (Bylund-Fellenius *et al.*, 1981; Hogan *et al.*, 1992a:1992b). For the same $\dot{V}O_2$, an increase in arterial PO_2 will have the reverse effect on the substrates of oxidative phosphorylation. Thus for the same rate of oxidative phosphorylation, the substrate concentration can be very different. Since [ADP], [Pi], [NADH], and [H^+] respond to the change in mitochondrial PO_2 to maintain a given $\dot{V}O_2$, the rate of flux of O_2 into the cell is being regulated. For a higher FIO_2, there is a smaller increase in [C] and decrease in [CP]. This would suggest that the flux within CP shuttle would be less compared with normoxia and hypoxia– yet the rate of oxidative phosphorylation and ATP hydrolysis is the same. Four possible mechanisms may explain this discrepancy of the same high energy phosphate flux with different

concentration gradients. First, another mechanism, other than diffusion, and not dependent on a concentration gradient, is affecting the exchange of high energy phosphates between the mitochondrion and the cross-bridge. Second, at a higher FIO_2 and the same $\dot{V}O_2$, competition between glycolysis and oxidative phosphorylation for substrate to phosphorylate may be less. Therefore, the rate of high energy phosphate turnover may be maintained with a smaller substrate concentration gradient. Third, the high energy phosphate flux is the same and it is the percent gradient of a cytosolic concentration (see Kammermeier, 1987) of an oxidative phosphorylation substrate which is now greater in the hyperoxic condition. If a higher percent gradient can be maintained for a given concentration of oxidative phosphorylation substrate or product then a higher maximal flux may occur as is observed in hyperoxia. Fourth, more ATPase and/or ATP synthase sites may be active or up-regulated (Das and Harris, 1989). This will reduce the concentration gradient necessary for a given flux of substrate.

Under normal *in vivo* conditions, at least for skeletal muscle, the answer lies somewhere between the two extremes of only convection or only diffusion regulating the rate of O_2 utilisation. This is why there is always a change observed both at the central site (*e.g.*, increase in cardiac output) and the peripheral site (*e.g.*, a change in a peripheral modulator of O_2 utilisation) when work rate is increased. At the cellular level, O_2 flux from the capillary to the mitochondrion depends on the PO_2 in the capillary and the PO_2 in the mitochondrion. Delivery and intracellular processes both affect each end of the O_2 pressure head. For steady state conditions, an increase in delivery will increase both PcO_2 and PmO_2. Contrarily, if ATP demand increases with no concomitant change in delivery then intracellular processes will decrease PmO_2 and decrease mean PcO_2. Being dependent on both delivery and peripheral mechanisms for O_2 utilisation enables a greater O_2 flux into the cell than being dependent on either process alone. Since both convective and diffusive mechanisms are intimately involved in establishing the PO_2 gradient between the capillary and the mitochondrion then the time course of O_2 utilisation is dependent on both central and peripheral processes. A study which alters one mechanism, (*e.g.*, a reduction in PaO_2) and

observes a slower $\dot{V}O_2$ response to an increase in work rate is correct in concluding that a central mechanism regulates $\dot{V}O_2$ kinetics. However, extending the conclusion to say that a peripheral mechanism does not alter $\dot{V}O_2$ kinetics is incorrect and illogical based on the above discussion.

On a larger scale, ATP hydrolysis, high energy phosphate shuttling, mitochondrial respiration, and O_2 flux into the cell all appear to behave as a coordinated system. If there is a change in one component of this system, then other components of the system change in order to correct any imbalance in order to maintain steady state behaviour. If this cannot be accomplished, then fatigue will occur. In exercise physiology, the imbalance is usually caused by changing ATP hydrolysis but may also be caused by a disturbance in any other component such as a reduction in arterial PO_2.

Steady state oxygen consumption

The $\dot{V}O_2$ was lower in the both the trained and untrained leg post training compared with pretraining. Henriksson (1977) noted a lower $\dot{V}O_2$ in both the trained and untrained leg after training. The effect was greater for the trained leg and thus Henriksson (1967) reported a significantly lower $\dot{V}O_2$ in the trained compared with the untrained leg after training. Saltin *et al.* (1976) also commented on a lower $\dot{V}O_2$ value after one-legged training but their difference did not reach significance, perhaps due to their much smaller number of measurements (N = 13) than the present study (N = 75). The lower percentage of maximal effort required after training may reduce accessory muscle activation and thereby lower post $\dot{V}O_2$(ss). The lower HR after training in the trained leg was expected (Saltin *et al.*, 1976). Also expected was the lower HR from the trained leg compared with the untrained leg after training (Henriksson; 1977; Saltin *et al.*, 1976).

$FETO_2$ responses

The serendipitous finding of the $FETO_2$ time course is very interesting. It has been rarely reported in the literature. The reliable time delay and exponential increase observed was only because the work rate increase was so small that volitional ventilation was almost constant. Linnarsson (1974) noted a relatively constant end tidal PO_2 for 15-20 seconds after exercise

onset. He attributed this delay to the time it takes blood to reach the lungs from the working muscles.

F_ETO_2 is dependent on ventilatory rate and venous PO_2. It follows that if one of these 2 factors is held constant, then a change in F_ETO_2 will reflect a change in the other factor. Thus if ventilation is held constant, the F_ETO_2 change should mirror a venous PO_2 alteration. When the one-legged step work rate protocol was performed with constant hyperventilation, the F_ETO_2 signal was very reproducible from one step to the next. For the ramp work rate protocol, the linear nature of F_ETO_2 during an imposed constant ventilation was profoundly different from that of volitional ventilation. This suggests that venous PO_2 was constantly decreasing during the unsteady state increase in work rate. I am not aware of any studies that have measured venous PO_2 during ramp exercise. However, steady state values for increasing single leg work rate indicate the a-vO_2 difference increases quickly at low work rates, then increase much more slowly at higher work rates (Andersen and Saltin, 1985). For 2 legged upright cycling, the steady state a-vO_2 difference increases quite linearly right up to maximal exercise (Saltin *et al.*, 1968) and venous PO_2 decreases linearly with increasing heart rate up to maximal exercise (Pirnay *et al.*, 1972).

The use of constant hyperventilation may provide a useful non-invasive measure of venous PO_2, if it can be validated against simultaneously recorded venous blood PO_2 measurements.

6.3 EXPERIMENT 4-GLYCOGEN DEPLETION AND $\dot{V}O_2$ TAU

Introduction

Substrates entering the TCA cycle are principally provided by 2 pathways: glycolysis and fatty acid beta-oxidation. It is possible that $\dot{V}O_2$ kinetics are affected differently by the two different fuel sources. It is often thought that carbohydrate utilisation has a very fast response and lipid

catabolism has a much slower response. Thus a hypothesis could be developed speculating that the $\dot{V}O_2$ tau from carbohydrate metabolism is much faster than the $\dot{V}O_2$ tau from lipid oxidation. Yet if there was no carbohydrate oxidation, $\dot{V}O_2$ tau would depend only on lipid sources. Without flux through the glycolytic pathway there would be a major reduction in the size of the O_2 deficit. If the O_2 deficit is reduced then $\dot{V}O_2$ tau must be shorter. This is implicit in the equation:

$\tau = O_2 \text{ deficit} / \Delta\dot{V}O_2(ss)$

DiPrampero *et al.* (1989) noted as an undocumented observation that the $\dot{V}O_2$ response was very fast in subjects with McArdle's disease. A possible explanation for the faster $\dot{V}O_2$ response when glycolytic activity is reduced is that glycolysis and mitochondrial respiration compete for the same substrate. This substrate could be ADP or creatine. Whatever the competing substrate, the relative amount of anaerobic glycolytic power to mitochondrial oxidative power may determine the time-dependent rate of O_2 utilisation. When glycolytic power is reduced, such as in the glycogen-depleted state, the changes in ADP, creatine, and CP may be less for a given submaximal work rate. This may occur because it will take a smaller concentration gradient to generate a given flux between the myofibrils and the mitochondrion when competition for substrate required by the mitochondrion is reduced.

The purpose of this investigation was to examine the effect of glycogen depletion on $\dot{V}O_2$ kinetics.

Methods

Six healthy male subjects completed this experiment. They were informed of the risks attendant upon the procedures to be followed and read, understood, and signed an informed consent. Subject characteristics are listed in Table 6-5. Each subject's $\dot{V}O_2$max and cardiorespiratory response was determined from a ramp ergometer exercise test with a 1 W • 2 s^{-1} ramp slope.

TABLE 6-5. *Characteristics of subjects.*

Subject	Age	Height (cm)	Weight (kg)	$\dot{V}O_2max$ ($l \cdot min^{-1}$)
1	39	186	86	4.25
2	25	187	80	5.26
3	22	184	75	4.96
4	22	179	70	3.63
5	26	168	64	4.32
6	27	178	67	3.68

Glycogen depletion in each subject was produced by a series of 90 minute ergometer rides at 50 % $\dot{V}O_2max$ to completion or to the limit of a subject's volition. The first 90 minute test was followed 15 minutes later by a series of 110 % $\dot{V}O_2max$ efforts each lasting 1 minute with a 2 minute rest. These 1 minute interval rides were performed until the subject could no longer maintain the power output for 1 minute. Two hours after completion of the high intensity rides, the subject then performed another 90 minute 50 % $\dot{V}O_2max$ ergometry test. After an overnight restricted diet (fat-protein only) the subject performed a third 50 % $\dot{V}O_2max$ ergometry test for 90 min or until volitional exhaustion.

$\dot{V}O_2$ kinetic determination

After the ramp test and 2 days prior to the first 90 min ergometry test, the subject performed a series of alternating step work rates to determine $\dot{V}O_2$ tau. This kinetic test consisted of alternating the work rate between 30 W and 80 W at 5 minute intervals for a duration of 45 min. This test generated a series of 4 step increases. This test was also performed 2 hours after the last 90 ergometry test.

Data analysis

Data for each 5 min ON-step were interpolated every second and then all 4 ON-steps were ensembled and the median value was chosen to form a single composite $\dot{V}O_2$ response for each subject, for each condition. Phase 1 of the step response was omitted and the remaining data were fitted with a one component exponential model.

Statistical analysis

The $\dot{V}O_2$ time constants derived from modeling were compared before and after glycogen depletion with a correlated *t* test.

Results

The glycogen-depleted condition did not alter the $\dot{V}O_2$ time constant for the 30-80 W step increase used in the study (p=0.20, two-tailed) (Table 6-6).

TABLE 6-6. *The oxygen utilisation time constant for the normal glycogen and glycogen reduced condition.*

	$\dot{V}O_2$ Time Constant (s)	
Subject	Normal Glycogen	Reduced Glycogen
1	30.2	33.4
2	19.0	17.2
3	27.2	26.6
4	30.6	33.8
5	45.2	46.0
6	28.8	37.8
Average	30.2	32.5
S.D.	8.5	9.8

S.D. is the standard deviation.

Discussion

The $\dot{V}O_2$ time constant for an ON-step to a light work rate was not altered by the glycogen-depleted condition. In general, a glycogen-depleting work rate protocol does not deplete glycogen but rather reduces it (*e.g.*, Grisdale *et al.*, 1990; Heighenhauser *et al.*, 1983). Thus glycogen reduction would be a more appropriate term to use. It has been shown that, for short term intense exercise, glycogen reduction does not alter performance nor glycogenolytic and glycolytic catabolism (Spencer and Katz, 1991). Similarly, above normal muscle glycogen concentration does not alter glycolysis during short term maximal exercise (Bangsbo *et al.*, 1992). If a limitation of glycogen supply were to modulate $\dot{V}O_2$ kinetics, then glycogen concentration would probably have to be reduced to levels which would be difficult to attain and perhaps deleterious to the subject.

Another possible reason for not observing an effect of glycogen reduction on the $\dot{V}O_2$ time course is that glycogen supply plays a limited role in $\dot{V}O_2$ kinetics for work rates below the lactate threshold. Therefore, the manner in which $\dot{V}O_2$ tau was determined in this study may not be sensitive enough to detect a change in glycogenolytic rate. Using a work rate increase to above the lactate threshold may involve proportionally more glycogenolytic activity in which case a glycogen reduction effect on the $\dot{V}O_2$ time course may be observed. However, the subject would not be able to perform more than one repetition while in the glycogen reduced state used in this study (Walsh and Banister, unpublished observation).

It has been shown that the ability to perform repetitive 25 s isometric contractions at 50 % MVC subsequent to glycogen reduction may not be due to the glycogen reduction but rather to another factor associated with the work protocol required to establish significant glycogen reduction (Grisdale *et al.*, 1990). Whatever the cause or causes of the reduced performance, such as attenuated neural drive or contractile force generation, it is clear that the debilitating mechanism did not alter the time course of oxygen utilisation in the present study. From this perspective, it

appears that the fatigue was not due to an attenuation of energy generating mechanisms unless two opposing factors were effecting $\dot{V}O_2$ tau concurrently. Perhaps the reduced glycogen decreased $\dot{V}O_2$ tau but fatigue within the energy generating processes increased $\dot{V}O_2$ tau. Such a determination cannot be made from the present study.

7.0 THE EFFECT OF A HETEROGENEOUS MUSCLE COMPARTMENT ON MODELED $\dot{V}O_2$ KINETICS

7.1 REVIEW OF LITERATURE

The $\dot{V}O_2$ response to a WR stimulus both below and above the lactate threshold is probably not a simple first order system (Table 5-1). Various approaches have been used in an attempt to describe the $\dot{V}O_2$ response better. The advent of small computers capable of thousands of calculations per second enables the application of a 10th order polynomial equation to the solution of the $\dot{V}O_2$ response. This process generates a very small error term. However, it must be asked "What are the physiological correlates of a 10th order polynomial?" If a model contains parameters that do not have any physiological relevance it should be considered invalid. A model of this nature, in only rare instances, provides any insight into seeking a hitherto unforeseen physiological correlate of a model parameter. Another question which must be asked is whether this approach is more valid than using a 9th order polynomial since there may be only a small reduction in the error term by choosing a higher numbered polynomial.

The time course of the $\dot{V}O_2$ response has often been modeled with a single component exponential equation (see section 2). To improve upon this model investigators have incorporated more parameters and/or components into this basic model. One approach to modeling the $\dot{V}O_2$ response to a step increase in work rate incorporates a time delay (td):

$$\Delta\dot{V}O_2(t) = \Delta\dot{V}O_2(ss) \cdot [1 - e^{-(t \pm td) / \tau}] \quad \text{.....(7-1)}$$

Physiologically, the time delay corresponds to the O_2 signal travelling from working muscle to the lungs and then to the mouth where it can be measured. If the distance between the locus of O_2 measurement and the locus of O_2 utilisation is reduced or if venous return is increased, then the time delay will be shorter.

Mathematical analysis of the single exponential equation indicates that equations without a time delay produce better goodness of fit (Morton, 1987). Morton (1987) concluded that if a time delay is incorporated into a single exponential model (as many authors have done), it contradicts the very properties it is designed to characterize. However, other authors have found that incorporating a time delay markedly improves modeling of the $\dot{V}O_2$ response to a step increase in work rate (Linnarsson, 1974; Whipp *et al.*, 1982). Whipp *et al.* (1982) have demonstrated that perhaps the best method to model $\dot{V}O_2$ kinetics at the onset of exercise is to model only the phase 2 response. Thus the data for the first ~20 s of exercise are omitted from the curve fitting procedure. This method has proved superior to other single component models both incorporating and omitting a time delay. Moreover, the latter is the only model able to produce similar time constants for step work rate increases from rest to 100 W and 0 W to 100 W.

Another approach to improve mathematical definition of the $\dot{V}O_2$ response time course is to describe it as a two component first order system instead of a single exponential first order system:

$$\Delta\dot{V}O_2(t) = \Delta\dot{V}O_2(ss)_1 \cdot [1 - e^{-t / \tau_1}] + \Delta\dot{V}O_2(ss)_2 \cdot [1 - e^{-t / \tau_2}] \quad \text{.....(7-2)}$$

Various physiological rationales have been proposed to justify the second component characterization. These rationales often apply to phase 4 kinetics and include additional metabolic activity produced by a temperature increase, an increase in circulating catecholamines, stimulatory effects of an increase in circulating potassium, and an additional O_2 cost of ventilation during exercise. The gain of the second component response is less than 10% of the first component and thus may be obscured to a degree by a large signal-to-noise ratio prevalent at lower work rates (Lamarra *et al.*, 1983).

A third approach to modeling the $\dot{V}O_2$ response is to combine both of the approaches described above, *i.e.*, to incorporate two first order components and have a time delay associated with each:

$$\Delta\dot{V}O_2(t) = \Delta\dot{V}O_2(ss)_1 \cdot [1 - e^{-(t \pm td) / \tau_1}] + \Delta\dot{V}O_2(ss)_2 \cdot [1 - e^{-(t \pm td) / \tau_2}] \quad \text{.....(7-3)}$$

Hughson and his co-workers commonly use this two component model with time delays to fit the $\dot{V}O_2$ response (Hughson and Inman, 1986; Hughson *et al.*, 1988). They have also found that incorporating a second order component significantly reduces the time constant of the first order component. Lamarra *et al.* (1983) found the same effect when they included a second order component to model the $\dot{V}O_2$ response to a work rate below lactate threshold. Although Hughson and co-workers and Lamarra *et al.* (1983) both used a two component exponential model to describe the $\dot{V}O_2$ response to a step work rate increase to a work rate below the lactate threshold, they applied the model components to different parts of the $\dot{V}O_2$ response curve. Hughson and co-workers assigned one component to the phase 1 response and one component to the phase 2 response. Lamarra *et al.* (1983) omitted the phase 1 response from their curve fitting procedure and assigned both components to the phase 2 response. Lamarra *et al.* (1983) demonstrated that O_2 kinetics were better described by a two component exponential equation with the second component having a time constant of about 2 minutes and accounting for ~5-7% of the $\Delta\dot{V}O_2(ss)$. Furthermore, the inclusion of a second order factor reduced the first order $\dot{V}O_2$ time constant from 43 s to 32 s. These conclusions were determined from exercise studies below the lactate threshold work rate. Certainly, at work rates above lactate threshold, the amplitude of the second component will be larger due to a more pronounced phase 4 effect. The phase 1 response has considerable variability under different working conditions. Thus it is perhaps best to follow the recommendation of Whipp and Ward (1990) and omit the phase 1 region of the $\dot{V}O_2$ response when estimating $\dot{V}O_2$ tau.

Caution must be taken when determining the unsteady state $\dot{V}O_2$ response from models that contain many parameters. Henry (1951) pointed out that the parameters are mathematically independent. However, when performing least squares curve fitting, the parameters are not independent. As the number of parameters increase the probability of parameter redundancy increases despite a reduction in the error term (Lamarra *et al.*, 1987).

A fourth method to model the $\dot{V}O_2$ response to exercise is to incorporate a single exponential term with a linear term. This linear term is added to explain the phase 4 $\dot{V}O_2$ response observed at a work rate greater than the lactate threshold (Armon *et al.*, 1991; Casaburi *et al.*, 1989a):

$$\Delta\dot{V}O_2(t) = \Delta\dot{V}O_2(ss) \cdot [1 - e^{-t/\tau}] + s \cdot t \quad \text{.....(7-4)}$$

where s is the slope of the $\dot{V}O_2$ response between 3 and 6 min of a step increase in work rate.

In contrast to the empirical $\dot{V}O_2$ models discussed above, structural $\dot{V}O_2$ models have been created in an attempt to describe and understand the $\dot{V}O_2$ response better. Margaria (1976) considered $\dot{V}O_2$ to be the result of energy supply and proposed an appropriate muscle model based on this hypothesis. This "peripheral" model consists of 3 hydraulic compartments: alactic, lactate, and aerobic energy generating compartments. Each energy system or compartment is represented by a tank which empties into a common drain. The flow through the drain represents ATP supply. The relative level in each of the 3 tanks determines the pressure head or driving force for their specific energy contribution into the common output. In Margaria's model, $\dot{V}O_2$ kinetics are regulated peripherally and active muscle is modeled as a homogeneous entity. No influence of blood flow on $\dot{V}O_2$ kinetics is considered. Morton (1984; 1985; 1986) extended and refined Margaria's model (referred to as the Margaria-Morton model) but the limitations mentioned above remain the same.

Barstow and co-workers have modeled the $\dot{V}O_2$ response from a different perspective than that represented by the Margaria-Morton model (Barstow and Mole, 1987; Barstow *et al.*, 1990). These investigators incorporated cardiac output ($\dot{Q}$) into the model as well as the working muscle oxygen uptake ($m\dot{V}O_2$). The time course of the $\dot{V}O_2(t)$ response was calculated from:

$$\dot{V}O_2(t) = \dot{Q}(t) \cdot [CaO_2 - CvO_2] \quad \text{.....(7-5)}$$

where

$$CvO_2 = CaO_2 - [\Delta\dot{V}O_2(ss) \cdot [1 - e^{-t/\tau m\dot{V}O_2}]] / [\Delta\dot{Q}(ss) \cdot [1 - e^{-t/\tau \dot{Q}}]] \quad \text{.....(7-6)}$$

CaO_2: concentration of O_2 in arterial blood ($ml \cdot 100\ ml^{-1}$).

CvO_2: concentration of O_2 in venous blood (ml·100 ml^{-1}).

$\dot{Q}(t)$: cardiac output (ml·min^{-1}) at time t (s).

$\Delta\dot{Q}(ss)$: the change in cardiac output (ml·min^{-1}) from baseline to steady state.

$\Delta\dot{V}O_2(ss)$: the change in oxygen uptake (ml·min^{-1}) from baseline to steady state.

$\tau\,\dot{Q}$: time constant (s) of cardiac output.

$\tau_m\dot{V}O_2$: time constant (s) of O_2 uptake by the muscle compartment.

With this model, Barstow and co-workers are able to determine the effect of various perturbations of cardiac output kinetics and muscle O_2 kinetics on the $\dot{V}O_2$ response measured at the lung. Like the Margaria-Morton model, the model developed by Barstow and co-workers assumes a homogeneous muscle.

The assumption that the muscle is a homogeneous tissue simplifies the complexity of the *in vivo* response. Although human skeletal muscle recruited in cycle ergometry is undeniably heterogeneous (*e.g.*, in capillarity, structural proteins, and respiratory enzymes), it is not known to what extent this heterogeneity influences the $\dot{V}O_2$ response. The heterogeneity occurs mainly between muscle units and not within a single muscle unit. As work rate increases, more muscle units are recruited. Muscle unit recruitment is an orderly process. Under physiological conditions, motor unit recruitment proceeds in a size related manner from small to large muscle units (Henneman, 1957; Henneman *et al.*, 1965). Thus it is logical to consider the muscle unit as the basic unit of whole muscle oxygen uptake ($\dot{Q}O_2$). The $\dot{Q}O_2$ response to a work rate change may be considered the sum of the individual responses of each muscle unit recruited to meet the power demand of the work rate. In order to generate a $\dot{Q}O_2$ response, the PO_2 gradient between the blood and the mitochondrion must increase. An increase in the concentration gradient of the modulator(s) of cellular respiration between the sites of ATP utilisation and the sites of O_2 utilisation must first be established. This is accomplished by an increase in ATP hydrolysis for crossbridge cycling and ion pumping. Consequently, more substrate is presented to the

mitochondrion for oxidation, thus more O_2 will be used thereby lowering mitochondrial PO_2 and increasing the O_2 concentration gradient from the blood to the mitochondrion. This, in addition to increased perfusion, will enhance O_2 flow into the muscle cell. In this way the $\dot{Q}O_2$ response is initiated. The rate at which $\dot{Q}O_2$ increases to a steady state is dependent on numerous factors. It is logical to assume that the shorter the diffusion distance between the crossbridges and the mitochondrion and between the mitochondrion and the blood, the faster the $\dot{Q}O_2$ response will be. For example, if the distance between the mitochondrion and the capillary is reduced, it will take a smaller O_2 gradient between the capillary and the mitochondrion to maintain a given O_2 flux into the muscle cell. The faster $\dot{V}O_2$ response induced by training (Hickson *et al.*, 1978) may be due to an increase in mitochondrial density and capillarity reducing the diffusion distance in trained muscle.

A small muscle unit has a greater mitochondrial content, more capillaries, and shorter diffusion distances associated with it than does a larger muscle unit and thus a smaller unit would be expected to have a faster O_2 utilisation time constant. $\dot{Q}O_2$ tau will be longer for a larger step change in WR. The greater demand placed on working muscle to meet the power output required of a larger WR induces the muscle to recruit more muscle units. These additional muscle units will be larger and therefore have less capillarity and mitochondrial content than muscle units recruited at a lower WR. Thus the $\dot{Q}O_2$ response of these larger muscle units will be longer because of their greater diffusion distances for O_2 and oxidative regulators. The overall muscle $\dot{Q}O_2$ response will therefore be slower at a higher WR because the mean muscle unit time constant will be longer.

This simple $\dot{V}O_2$ model, outlined above, is able to explain seemingly contradictory data. Under hypoxic conditions $\dot{V}O_2$ tau measured during a step change in WR is longer than under normoxic conditions, yet there is no change in diffusion distances. In fact, what has changed is the O_2 pressure head from the blood to the mitochondrion. Maintenance of a given O_2 flux into working motor units, when PaO_2 is reduced, will require a larger respiratory regulator gradient to be established between the crossbridges and the mitochondria. This will reduce the PO_2 at the

mitochondria, compensating for the reduced PO_2 in the blood. This compensation maintains the PO_2 difference, and thus O_2 flux, between the blood and the mitochondria. Since the gradients required are larger when PaO_2 is reduced, it will take more time for $\dot{V}O_2$ to reach steady state. This is analogous to the longer $\dot{V}O_2$ kinetic response at a higher work rate because a greater percentage of $\dot{V}O_2max$ is required for both conditions. The hypothesis developed here predicts that for a given work rate, the concentration of any stimulator(s) of cellular respiration such as creatine will be greater under hypoxic conditions. Linnarsson *et al.* (1974) have reported larger changes in [CP], [C], [ADP], and [Pi] when similar work is performed in hypoxia than in normoxia.

An alternative interpretation of the different $\dot{V}O_2$ response to the hypoxic condition compared with normoxia is that for a given work rate more muscle units are recruited in hypoxia. Such an effect has been observed during occlusion (Moritani *et al.*, 1992). In line with the present interpretation, each additional muscle unit recruited would have larger diffusion gradient as previously noted. This may occur because the larger O_2 regulator gradient established for the hypoxic condition will cause faster carbohydrate oxidation. Recruiting more motor units, but at a lower frequency, may have a glycogen sparing effect. This hypothesis requires the existence of a metabolic feedback system influencing motor unit recruitment. There is experimental support for this under some physiological conditions (Bigland-Ritchie, 1986; Bigland-Ritchie *et al.*, 1986b), but direct unequivocal investigation using hypoxia has not been performed. The recruitment of additional motor units during hypoxia, where each additional motor unit has a longer time constant than a previously recruited unit, will increase whole body $\dot{V}O_2$ tau. This is analogous to the observation of a longer $\dot{V}O_2$ time constant associated with a larger step increase in work rate.

7.2 EXPERIMENT 5-SIMULATION OF MUSCLE UNIT $\dot{Q}O_2$ TAU HETEROGENEITY

Introduction

The oxygen uptake time course to an increase in work rate has been modeled by various investigators (Barstow and Mole, 1987; Barstow *et al.*, 1990; Margaria, 1976; Morton, 1984; 1985; 1986). In each of these models, the muscle compartment is homogeneous with respect to its biochemical and physiological properties. For these models, the basic unit of the muscle oxygen uptake response ($\dot{Q}O_2$) is the entire muscle. However, it is well known that a muscle may consist of hundreds of muscle units. Within a single muscle, these units display a broad spectrum of physiological and biochemical characteristics which determine the rate of metabolic response to an increase in energy demand. Thus the muscle unit and not the entire muscle is the basic unit of the $\dot{Q}O_2$ response.

To generate an increase in oxygen utilisation within a muscle unit, there must be an increase in the cross-bridge turnover thus increasing ATP utilisation. This will increase the presentation of substrate to the mitochondrion, generating an increase in mitochondrial oxygen utilisation which will lower mitochondrial PO_2. This increases the O_2 gradient between the mitochondrion and the blood. This larger gradient and an increase in perfusion will augment the rate at which the muscle consumes oxygen. In this way a $\dot{V}O_2$ response is initiated. The rate at which $\dot{V}O_2$ increases to a steady state may be dependent on numerous factors. It is logical to assume for instance that the shorter the diffusion distance between the crossbridges and the mitochondrion and between the mitochondrion and the blood, the faster the $\dot{V}O_2$ response will be.

A small muscle unit has a greater mitochondrial density, more capillaries, and shorter diffusion distances associated with it than does a larger muscle unit (Saltin and Gollnick, 1983)). Thus a smaller muscle may have a faster rate of oxygen utilisation during the unsteady state. Kushmerick *et al.* (1983) have shown that the rate of change of phosphocreatine and inorganic phosphate is

faster in cat soleus (predominantly slow twich fibres) than in cat biceps (predominantly fast twitch fibres) muscle both at the beginning of stimulation and at recovery. Both the rate and magnitude of phosphocreatine change induce by a step increase in work rate are closely related to the rate and magnitude of the change in oxygen consumption in skeletal muscle (Mahler, 1985). These data suggest that the rate of oxygen utilisation may be related to muscle fibre type. Even in a homogenous tissue such as the heart, the oxygen uptake response time is longer when the step increase in metabolic demand is larger (Eijgelshoven *et al.*, 1992).

The purpose of this study is to use the $\dot{V}O_2$ model of Barstow and co-workers (Barstow and Mole, 1987; Barstow *et al.*, 1990) modified by a muscle compartment which is subdivided to represent the muscle as several heterogeneous muscle units rather than as a homogeneous entity. Each muscle unit will be assigned a different time constant of oxygen utilisation and the effect this will have on the overall pulmonary $\dot{V}O_2$ response is examined.

Methods

The model used to calculate the $\dot{V}O_2$ response to a change in work rate was that described by Barstow and Mole (1987). This model was modified to incorporate a heterogeneous muscle compartment consisting of 377 muscle units. For each simulation, every muscle unit was assigned a different $\dot{Q}O_2$ time constant. For different simulations of the model response a different time constant was assigned to each muscle unit. Five different profiles of time constants for the entire muscle were used (Fig. 7-1).

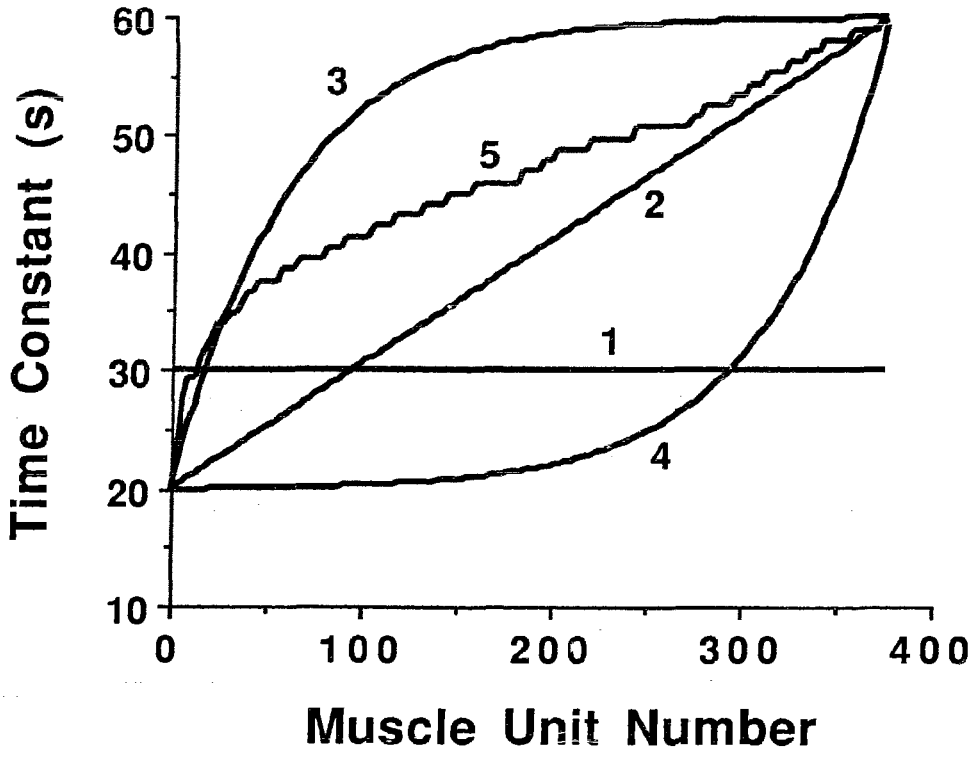

Fig. 7-1. The $\dot{Q}O_2$ time constant applied to each muscle unit for each of 5 different time constant profiles used. See text for a further description.

Profile 1 was the control in which the $\dot{Q}O_2$ time constant was the same for each muscle unit, *i.e.*, the muscle was modeled as a homogeneous compartment. Profiles 2, 3, and 4 were generated mathematically to perturb the muscle $\dot{Q}O_2$ response in various ways. Profile 2 simulated a linear increase in the $\dot{Q}O_2$ time constant for successively recruited muscle units. Profile 3 was an exponential saturating trend and profile 4 was an exponentially increasing pattern of $\dot{Q}O_2$ time constants for each muscle unit. The time constant profiles were generated by the following formulae in which n is equivalent to the muscle unit number:

Profile 1: tau = 30 s for each muscle unit,.....(7-7)

Profile 2: tau = 40/377 · n + 20,.....(7-8)

Profile 3: tau = 40 · [1 - $e^{(-n/60)}$] + 20, and.....(7-9)

Profile 4: $\tau = 0.1 \cdot e^{(-n/60)} + 19.92$ .....(7-10)

The constant of 60 used in profiles 3 and 4 was to give a moderate rate of increase to the exponential functions. All the other constants were selected to constrain the tau values between 20 and 60 s.

No data in the literature exist concerning the $\dot{Q}O_2$ time constant for each muscle unit of a mixed muscle. Profile 5 is an attempt to predict how the $\dot{Q}O_2$ time constant may vary between muscle units in a physiological manner. Three steps were used to generate this profile:

(1) First it was assumed that the $\dot{Q}O_2$ time constant was proportional to the succinate dehydrogenase (SDH) content measured in various fibres of a single mixed muscle. Training does increase SDH activity and reduce the $\dot{V}O_2$ time constant (Hickson *et al.*, 1978) as well as alter a host of other physiological and biochemical processes at both central (Clausen, 1977) and peripheral loci (Saltin and Gollnick, 1983). This does not imply that the $\dot{Q}O_2$ time constant is regulated by SDH activity but rather a training induced change in SDH activity may be related to a mechanism regulating the time course of oxygen utilisation. The single fibre SDH activities of human tibialis anterior muscle of Reichmann and Pette (1982: their Fig. 3) were used to approximate the SDH distribution in the modelled muscle of the present experiment (Fig. 7-2). A fifth order polynomial was used to describe the relative SDH activity (Fig. 7-2) for each muscle unit (n):

$$\text{relative SDH} = 5.0489 - 4.525 \cdot e^{(-2n)} + 4.352 \cdot e^{(-4n^2)} - 2.2496 \cdot e^{(-6n^3)} + 5.4633 \cdot e^{(-9n^4)} - 5.0661 \cdot e^{(-12n^5)} \quad \text{.....(7-11)}$$

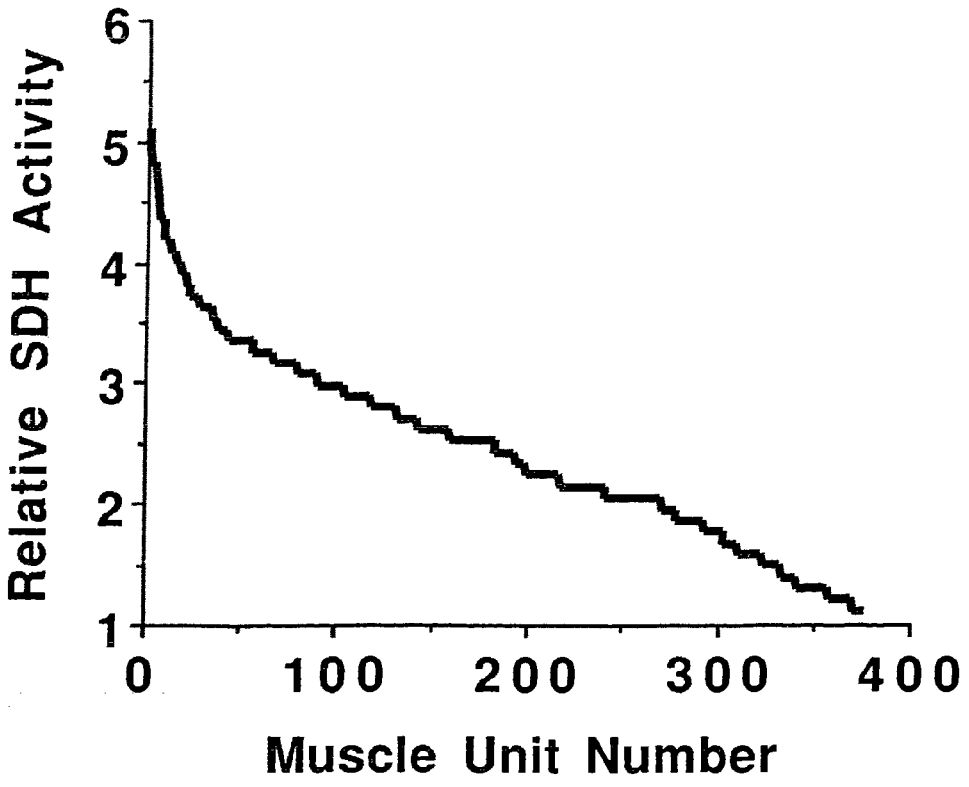

Fig. 7-2. The distribution of succinate dehydrogenase activity of single fibres of human tibialis anterior muscle.

(2) Currently there are no data relating the time course of O_2 utilization to SDH activity or mitochondrial content. In place of such a relation, data from Gollnick *et al.* (1985: their Fig. 4) were used. These authors related mitochondrial protein content to the delay time of O_2 utilisation under *in vitro* conditions (Fig. 7-3). The relative SDH activity data of Reichmann and Pette (1982) were used to represent the mitochondrial protein concentration of Gollnick *et al.* (1985) in the following manner:

[protein] = relative SDH • (0.8/5.5).....(7-12)

The constant 0.8 is the maximum protein concentration taken from Gollnick *et al.* (1985: their Fig. 4) and 5.5 is the maximum relative SDH activity taken from Reichmann and Pette (1982: their Fig. 3).

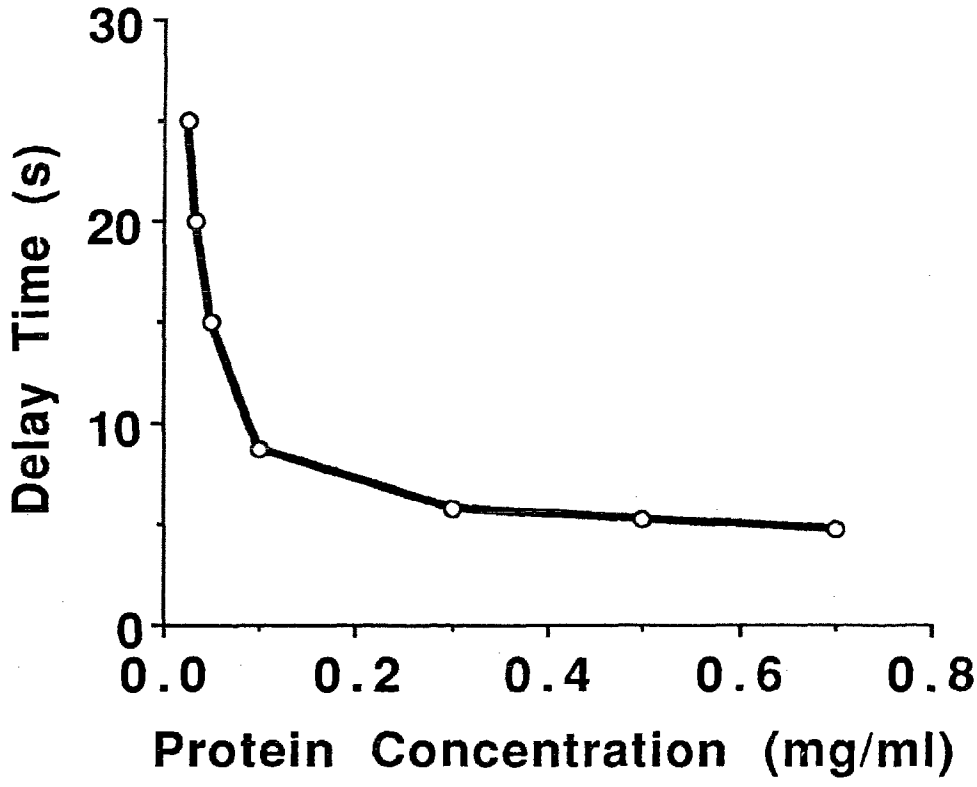

Fig. 7-3. The delay time of O_2 utilisation in a mitochondrial suspension as a function of mitochondrial protein concentration. Modified from Gollnick *et al.* (1985).

Having related the distribution profile of SDH activity to mitochondrial protein concentration, I then fitted the data of Fig. 7-3 with a power decay function to calculate time delay (s) as a function of protein concentration as follows:

$$\text{time delay} = 0.36 \cdot [\text{protein}]^{(-1.1)} + 4.4 \quad \text{.....(7-13)}$$

(3) The manner in which mitochondrial protein influences the time delay of O_2 utilisation (Fig. 7-3) was assumed to reflect the manner in which the $\dot{Q}O_2$ time constant may be

altered by a regulating variable. Thus the time delay of O_2 utilisation from the data of Gollnick *et al.* (1985) was converted in a linear process to the $\dot{Q}O_2$ time constant (tau: s) as follows:

Profile 5: tau = [(time delay * (-1) + 5.11) * 10] + 20.....(7-14)

The constants were chosen so as to constrain the tau values to between 20 and 60 s.

The minimum $\dot{Q}O_2$ time constant was set at 20 s. This is at the faster end of time constants usually observed in breath-by-breath human studies (Casaburi *et al.*, 1989a). The time constant of cardiac output response used for the $\dot{V}O_2$ model was 10 s. This value is similar to both the default value used by Barstow and Mole (1987) and that which has been determined from Doppler cardiography (Eirksen *et al.*, 1990).

In the model described, the $\dot{Q}O_2$ response of every recruited muscle unit was determined based on its individual time constant and summed to give a whole muscle $\dot{Q}O_2$ for each second. Modeling $\dot{V}O_2$ in this way attributes the same $\Delta\dot{Q}O_2$ to each muscle unit. *In vivo* muscle units vary greatly in size and thus vary in their ability to generate tension. Since the tension generated by a muscle unit determines its oxygen consumption a second variable of muscle unit dependent $\Delta\dot{Q}O_2$ was added to the muscle compartment. The tension of each muscle unit was generated in a physiological manner according to the motor unit recruitment algorithm of Wani and Guha (1975). In brief, the Wani and Guha recruitment algorithm models a muscle with 377 motor units, each unit having a successively larger twitch tension. A step increase in generated muscle tension includes both recruitment of additional motor units and an increase in firing frequency (and thus tension) of those motor units already recruited.

Recruitment of all 377 motor units of the Wani and Guha model generates a tension of approximately 2000 g each twitch (Fig. 7-4). Assuming the muscle is recruited at 1 Hz during cycling ergometry at 60 rpm, then the muscle has a power output of 2000 $g \cdot s^{-1}$. This was arbitrarily scaled to be equivalent to 300 W. Using the conversion of Barstow and co-workers of

1.0 l·min^{-1} O_2 uptake per 100 W. Thus muscle $\dot{Q}O_2$ demand to recruitment of all muscle units was 3.0 l·min^{-1}.

The muscle model of Wani and Guha (1975) was used to generate tension profiles for two different work rates. The tension profiles was based on the recruitment of all 377 motor unit and the recruitment of 200 motor units (Fig. 7-4).

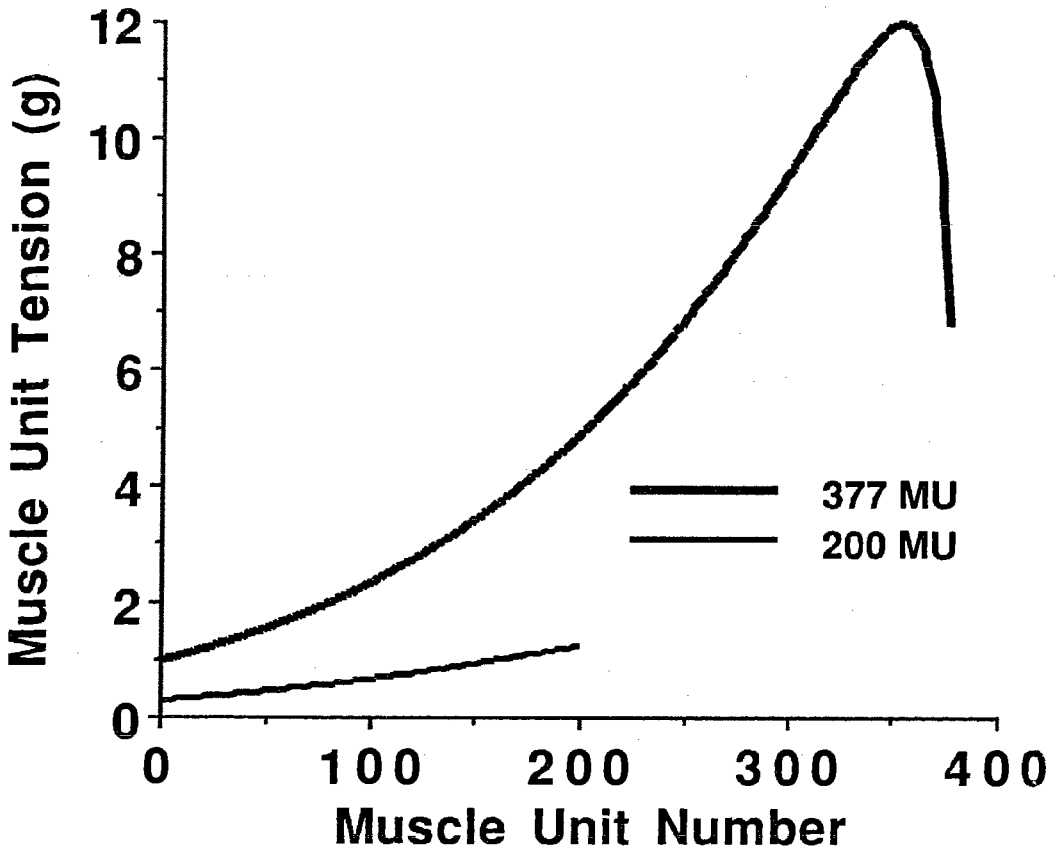

Fig. 7-4. The twitch tension generated by a muscle unit when 377 and 200 muscle units are recruited.

The total muscle tension generated when 377 muscle units are recruited is 2000 g. The muscle tension generated when 200 units are recruited is 150 g and this was equated to a work rate of

22.5 W and to a $\dot{Q}O_2$ of 0.225 l·min^{-1} thus keeping the conversions linear with those use for the recruitment of 377 muscle units.

A simulation to determine the pulmonary $\dot{V}O_2$ response to a step increase in work rate was performed for each of the 5 time constant profiles shown in Fig. 7-1 for both work rate conditions (rest to 22.5 W and rest to 300 W). The starting parameters for the $\dot{V}O_2$ model were the same as those used by Barstow and Mole (1987): venous volume=1040 ml; $\dot{V}O_2$ rest=50 ml·min^{-1}; CaO_2=0.21 ml·ml^{-1}. The change in cardiac output was set at 5 times that of the increase in $\dot{V}O_2$. The muscle unit $\dot{V}O_2$ response was determined from its assigned $\dot{V}O_2$(ss) and $\dot{V}O_2$ time constant according to Eqn. 7-1. The whole muscle oxygen consumption at any point in time was the sum of the individual muscle unit's oxygen consumption at the same point in time. This value was inserted into the muscle compartment portion of Eqn. 7-6.

Calculated pulmonary $\dot{V}O_2$ data (Eqn. 7-5), determined every second for 180 s, were curve fitted with either a 1 or 2 component exponential equation. Phase 1 was omitted prior to curve fitting. The curve fitting was done with a reiterative method. Reasonable ranges for time constant and steady state values were entered into the reiterative program and all parameter combinations were calculated. The parameter combination with the lowest sum of squares error term was selected as the best fit. All results were visually verified.

All modeling and curve fitting was done on a Macintosh II computer (Apple Computer, USA) using LabVIEW (National Instruments, USA) software.

Results

The pulmonary $\dot{V}O_2$ responses for each work rate and time constant profile are indicated in Fig. 7-5 and Fig. 7-6. The phase 1 response is much longer for the lower work rate step because the magnitude of the cardiac output response is much less.

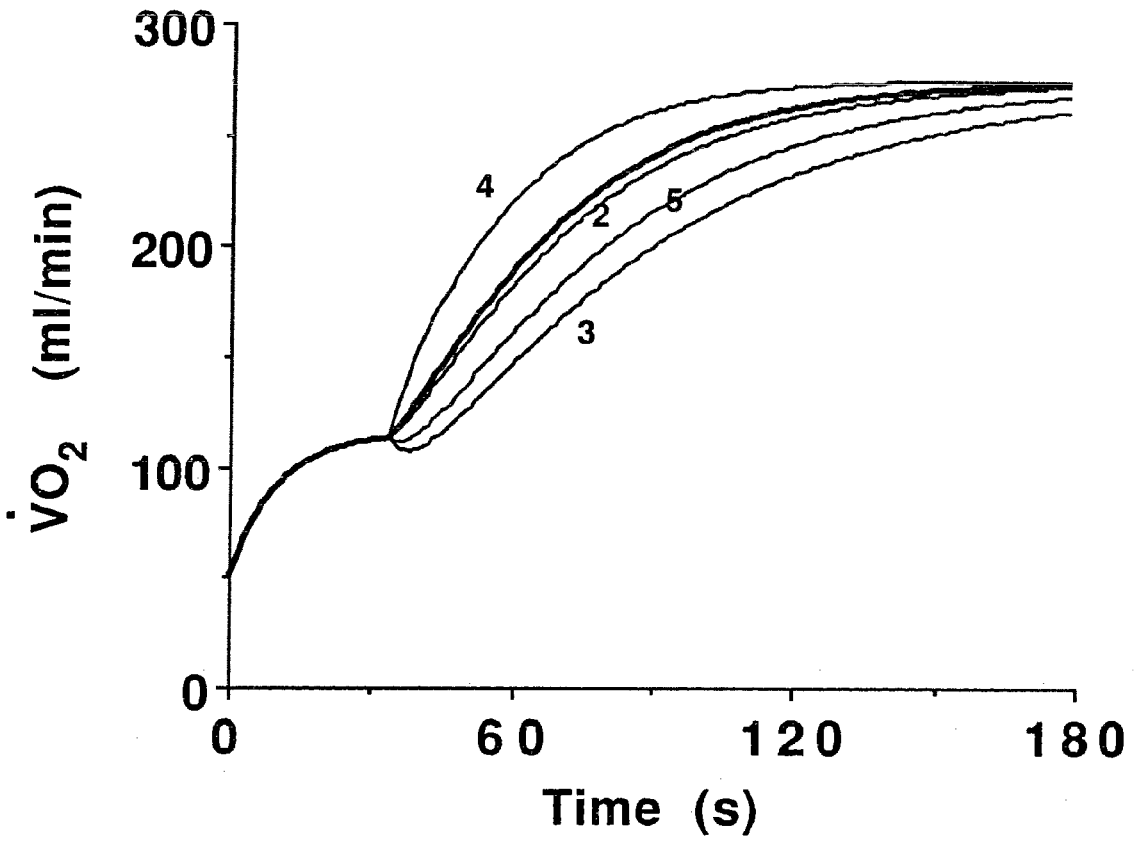

Fig. 7-5. Pulmonary $\dot{V}O_2$ responses obtained when the work rate was equated to 22.5 W (200 muscle units recruited) using different muscle unit time constant profiles. The $\dot{V}O_2$ response to profile 1 (control) is indicated by the thick line. $\dot{V}O_2$ responses from profiles 2, 3, 4, and 5 (thin lines) are indicated by number.

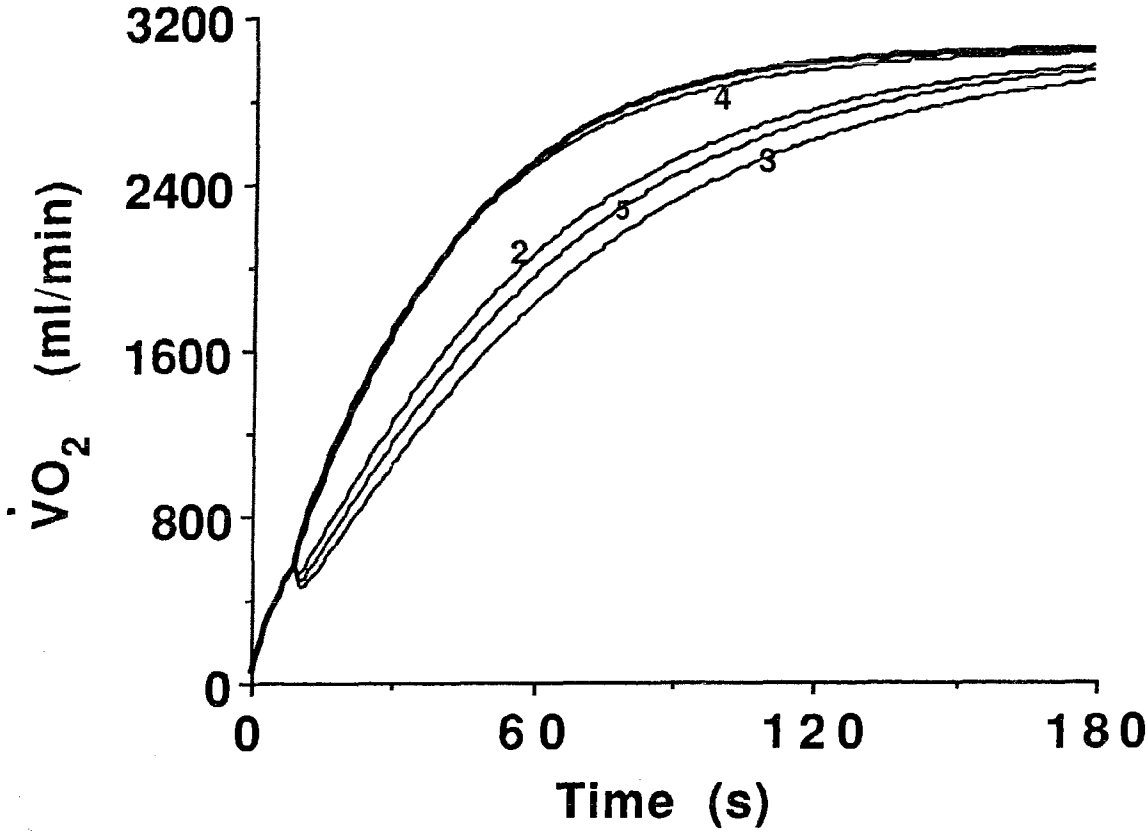

Fig. 7-6. Pulmonary $\dot{V}O_2$ responses obtained when the work rate was equated to 300 W (377 muscle units recruited) using the different muscle unit time constant profiles. The $\dot{V}O_2$ response to profile 1 (control) is indicated by the thick line. $\dot{V}O_2$ responses from profiles 2, 3, 4, and 5 (thin lines) are indicated by number.

Each $\dot{V}O_2$ time constant profile, except for the control, demonstrated a lengthening of the phase 2 $\dot{V}O_2$ time course when the step increase in work rate was 300 W compared with the 22.5 W step (Fig. 7-7).

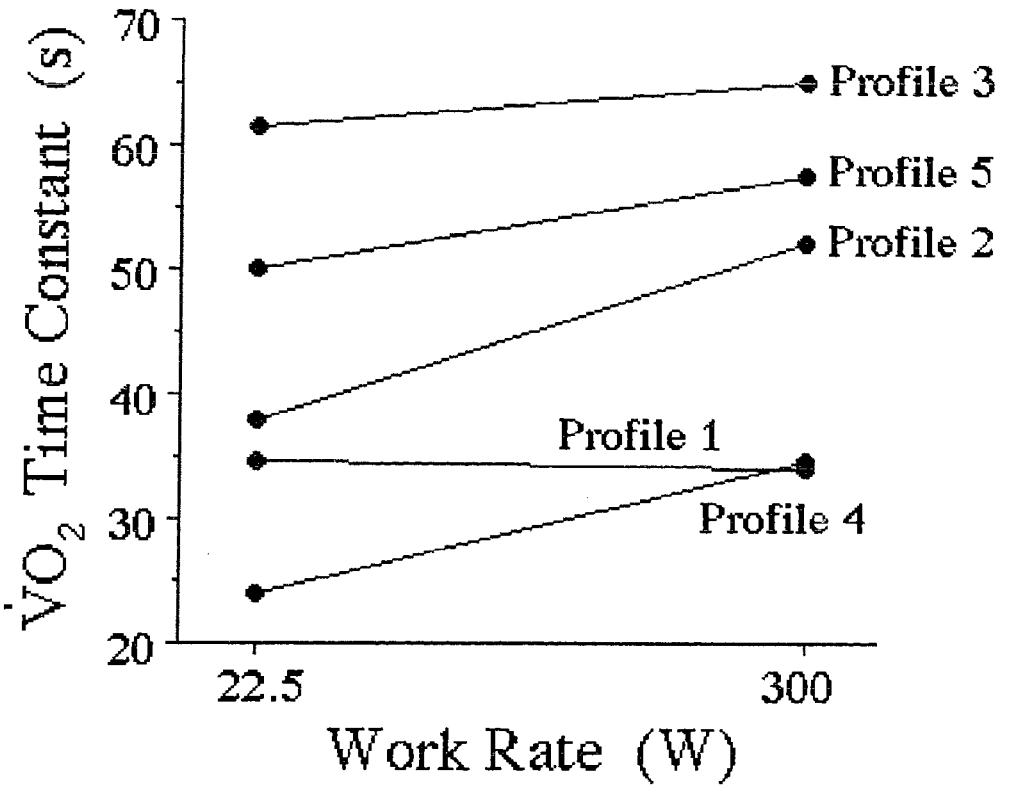

Fig. 7-7. Resultant pulmonary $\dot{V}O_2$ time constants at two points, 22.5 W (200 muscle units recruited) and 300 W (377 muscle units recruited) calculated from the contribution of recruited muscle units along the time constant profiles shown in Fig. 7-1.

The pulmonary $\dot{V}O_2$ responses were well fitted by a single exponential model (Fig. 7-8). There is no trend evident which would indicate a second component. As such, the two exponential component curve fitting did not significantly alter the error term of the single component fitting procedure.

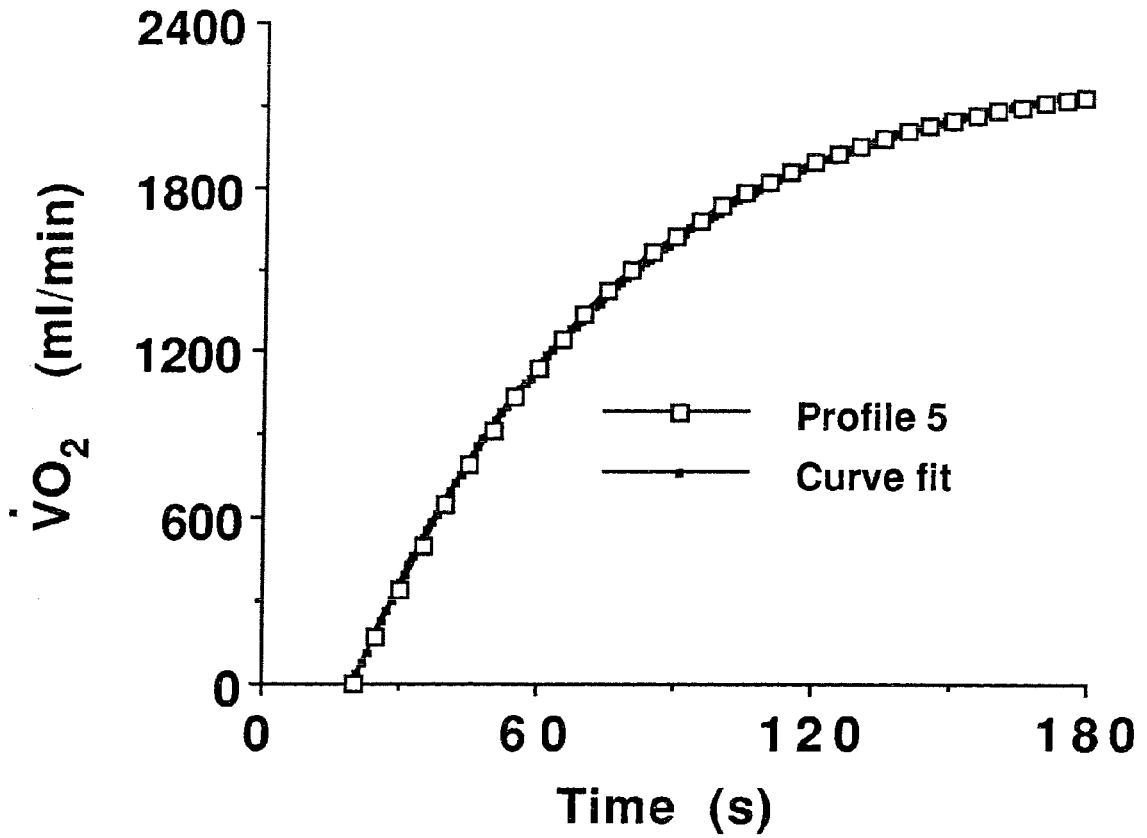

Fig. 7-8. The pulmonary $\dot{V}O_2$ response and curve fitted data for 300 W (377 muscle units recruited) with time constant profile 5. The first 19 s (phase 1) of the $\dot{V}O_2$ response were removed prior to curve fitting. The pulmonary $\dot{V}O_2$ response is the difference between 20 s and steady state. For clarity, only every fifth point of the $\dot{V}O_2$ response is shown.

Discussion

Whether the locus of $\dot{V}O_2$ kinetic regulation is central or peripheral is an on going debate (Hughson, 1990; Walsh, 1992; Whipp and Mahler, 1980). It has been shown (Casaburi *et al.*, 1989a; Hughson and Morrissey, 1982), although not always (di Prampero *et al.*, 1989; Whipp and Wasserman, 1972; Whipp *et al.*, 1982), that the $\dot{V}O_2$ time constant is longer when the step

increase in work rate is larger. Modeling the $\dot{V}O_2$ response with a heterogeneous muscle compartment has demonstrated a lengthening of the $\dot{V}O_2$ time course when the step increase in work rate was larger. Thus, the increase in the $\dot{V}O_2$ time constant observed for cycle ergometry when a larger work rate increase is used may be explained by the spectrum of physiological and biochemical diversity evident in human skeletal muscle.

Variation of muscle unit time constants within a given muscle does not change the pulmonary $\dot{V}O_2$ time course from resembling a single exponential. Therefore, if motor unit time constants and tensions change in a continuous manner, as in the present study, then any change from a single exponential of a $\dot{V}O_2$ time course would not be due to variable muscle unit time constants. Riggs (1963) demonstrated, using a semi-log plot and limited data points, that to distinguish 2 separate exponential and parallel processes, the time constants of the two components must vary by at least 3 fold. Although a much more sensitive curve fitting analysis was used in the current study, the continuous change of the muscle unit time constants precluded any separation of components. This illustrates the possibility that a measured exponential response may contain numerous parallel components which cannot be distinguished. Thus interpretations of such data, based on only a single component process, may be erroneous.

The slow rising component of $\dot{V}O_2$ commonly observed at a work rate above the lactate threshold (Casaburi *et al.*, 1987) has recently been shown to reside mostly within the muscle compartment (Poole *et al.*, 1991). This rules out several effects such as increased ventilatory work and increased cardiac work as a significant factor contributing to the slow component. The slow component was not evident with the simulation conditions used. Since the simulation data were noise free, even a slow component with a very small amplitude would have been evident by 180 s. To generate a continuing slow rise in $\dot{V}O_2$ with the present model would require a separate group of muscle units with a much slower time constant. A change in the efficiency of a sub population of muscle units would alter the amplitude but not the time course unless the efficiency change occurred sometime after exercise onset.

8.0 CONCLUSIONS

The mechanism regulating the time course of oxygen utilisation during an increase in work rate is neither central or peripheral. The increased O_2 flux demanded by the work rate increase requires either a greater PO_2 gradient or a greater O_2 delivery, or both. If $\dot{Q}$ does not change during a step increase in work rate then the PO_2 gradient must increase and this increase will be due to a lowering of the intracellular PO_2. This will result in a greater a-vO_2 difference. If $\dot{Q}$ changes to such a degree that all the $\dot{V}O_2$ increase demanded can be met by an increase in $\dot{Q}$ then the O_2 flux is increased by a relative increase in PcO_2 perhaps accompanied by a reduction in diffusion distance due to relatively more capillaries being perfused than muscle fibres recruited. This may not change the a-vO_2 difference. For an increase in $\dot{V}O_2$, the degree of change of intracellular PO_2 (and ΔCP also) will be inversely proportional to $\Delta\dot{Q}/\Delta$a-vO_2. If a WR increase induces both an increase in $\dot{Q}$ and a-vO_2, then the increase in the rate at which O_2 will diffuse into the muscle cell is dependent on the time course of establishing an decrease in intracellular PO_2 and a relative increase in PcO_2. Thus, for most physiological conditions, it is the interplay of both central and peripheral processes that establish the rate at which O_2 diffuses into working muscle for a step increase in work rate.

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